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**REPORT OF THE
NORTHERN REGIONAL RESEARCH CENTER**

May 1984

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PROGRESS REPORT
OF THE
NORTHERN REGIONAL RESEARCH CENTER
MAY 1984

INTRODUCTION

The Northern Regional Research Center is one of the major research facilities of the Agricultural Research Service, U.S. Department of Agriculture. The Center gives emphasis to post-harvest research to increase the use and cash value of farm products and by-products as well as to decrease losses during storage, processing, and transport due to intrinsic deterioration or external agents such as insects and microorganisms.

The resources of the Center are focused on three goals of importance to agriculture and the consumer:

- Ensured quality and safety of food for domestic and export markets
- Reduced dependence on nonrenewable resources
- Increased efficiency of plant production and protection

To meet these goals, research is organized to create knowledge or technology within the following programs:

- Protection of food from toxins
- The basis for reducing post-harvest losses
- Relationship between plant constituents and their functionality or nutritive value
- Microbial systematics and genetics
- Biotechnology
- Control of plant-pest interaction
- Regulation of biosynthesis in plants
- Beneficial plant-microbe associations

The fields of science undergirding these programs include microbial germplasm and ecology, regulation of plant and microbial metabolism, gene transfer and expression, chemistry and physics of natural polymers, and the molecular basis of functionality.

Much of the research involves crops of major importance to the north central region of the U.S., although plants of potential importance as a source of strategic materials or biomass are included.

The Center is organized into five Research Laboratories, each constituted of approximately 30 scientists plus technical support personnel. While some research programs are the exclusive province of a single Laboratory, others involve the participation by scientists in several Laboratories through formal and informal cooperation. The Northern Agricultural Energy Center is part of the Northern Center. Its studies on fermentation and chemical processes are done here while agronomic aspects are done at other ARS locations. The research is coordinated with that of its counterpart at Tifton, GA.

Scientists at the Northern Center cooperate with scientists at other ARS facilities, universities, state experiment stations, research institutions, industrial organizations, and other government agencies. Much of the cooperation is informal, but some work is done under cooperative agreements and memoranda of understanding. In addition, the research is supplemented by projects in foreign countries under the aegis of Public Law 480.

This report summarizes research done at the Center during 1983 and lists also the resulting publications and patents. The summaries include some preliminary results that have not been tested sufficiently to justify general release. Because of this, the report is not intended for publication and should not be referenced in literature citations. When adequately confirmed, such results will be released promptly through standard publication channels.

SELECTED ACCOMPLISHMENTS

Sumac, Potential Source of Industrial Chemicals, Alternative Crop. Smooth sumac (*Rhus glabra* L.) was identified at NRRC as a promising source of oil and phenolics, especially tannins. The initial specimen yielded 20 percent "polyphenol", 6 percent oil, and 7 percent protein. A cooperating ARS agronomist at Beltsville, MD, evaluating germplasm collected at 17 locations in 3 states, found that, although a perennial, sumac grows well from seed and can be harvested once during the planting year. Subsequent years should allow two harvests. Characteristics such as plant survival, vigor, dry matter yield, and number of secondary shoots showed significant genetic diversity as did polyphenol and oil contents. Significant increases in extractives contents and moderate improvement in yield should be possible by exploiting existing variability. [See Biomaterials Conversion Laboratory (BC), D.1.]

Interactions of Brans with Minerals in Human Diets. Measurements on brans prior to ingestion and after passage through the human GI tract reveal significant changes in mineral binding. Wheat bran remnants retrieved from human fecal matter contain more Ca than did the starting material and thus are able to act as a mineral sink to remove Ca from the GI tract. This observation is consistent with clinical observations that indicate excessive amounts of wheat bran are able to cause a negative calcium balance. Dry-milled corn bran has been shown to bind *in vivo* significantly more Ca, Cu, Fe, and Zn than it contained prior to ingestion. [See Cereal Science and Foods Laboratory (CSF), B.2.]

Practical Applications of Reversed-Phase High-Performance Liquid Chromatography for Analysis of Cereal Proteins. Reversed-phase high-performance liquid chromatography (RP-HPLC), an excellent analytical technique for cereal proteins, has now found many practical uses. Methods have been developed to rapidly extract and separate wheat gliadin proteins, and thereby identify varieties. Chromatographic conditions have been modified to improve the method's accuracy, and reduce analysis time to ten minutes. This ability to rapidly identify wheats may be used in commercial channels to identify varieties having specific quality characteristics, and to identify hybrids and specific varieties as a basis for use and value. Analysis of durum gliadins, as well as of bread wheat glutenin subunits, has also identified components associated with quality; RP-HPLC can thus be used by breeders and geneticists to screen for specific proteins or their subunits which are related to quality. RP-HPLC is also an excellent and rapid preparative method for isolation of cereal proteins. Furthermore, RP-HPLC is useful for corn and sorghum endosperm proteins; varieties may be identified and evolutionary relationships demonstrated. New computer techniques also permit identification and quantitation of components of hybrids and mixtures. Thus, RP-HPLC can be used to isolate, characterize, and compare cereal proteins and thereby predict, measure, improve, and assure good quality and value of all cereals and cereal-based products. (See CSF, C.1.)

Food Uses of Corn Distillers' Grains. As a result of a Congressional mandate in 1981, the USDA was directed to investigate potential food uses of protein-rich byproducts of alcohol production from grain, and to evaluate possible incorporation of these byproducts into foods for the "Food for Peace" program. In response, an interdisciplinary research team was formed to investigate this problem. Blended foods were formulated, with aid of a computer, which contained corn meal, soy flour, and nonfat dry milk (CSM), plus varied amounts of high-protein corn distillers' dried grains (CDG). All blends were formulated to contain 18% protein and optimum amounts of all essential amino acids, and to meet requirements for protein efficiency ratio and digestibility. A taste panel found reduced flavor acceptability for CDG-fortified CSM blends, but flavor improvement was attained through an extraction process. The fiber content of blends containing 10% CDG was excessive; therefore fiber content of CDG was reduced through dry milling and sieving. Because of these studies and improvements, it is probable that products containing up to 10% CDG can be prepared which may be acceptable in blended foods. (See CSF, C.2.)

Characterization of Metabolic Mutants Useful for Cloning/Cell Fusion Studies. This research is being conducted to develop specific yeast strains as hosts for genes required for pentose fermentation. The two primary enzymes involved in xylose utilization, xylose reductase and xylitol dehydrogenase have been identified. Specific cloning techniques are being employed to identify the genes/enzymes responsible for conversion of xylose to xylulose.

In developing cloning strategies for the transformation of the primary genes involved in pentose utilization, a number of selected mutants have been isolated being deficient in xylose utilization. The Pachysolen tannophilus PM 213 mutant is xylose negative/glucose positive and deficient in xylitol dehydrogenase activity but xylose reductase positive. Cell extracts of xylose induced PM 213 over-produces the reductase and xylitol as end product.

This mutant is most important as a host for gene restoration. The PM 213 mutant will be transformed with plasmids from a Pachysolen clone bank and assayed for xylitol dehydrogenase activity. [See Fermentation Laboratory (F), A.1.]

Demonstration of Key Enzymes in Pentose Metabolism. This investigation utilizes selective biochemical techniques to isolate and purify desired enzymes to use as probes for selective gene transformation. The first two enzymatic steps in the transformation of xylose by P. tannophilus have been defined. The NADPH-linked D-xylose reductase and NAD-linked xylitol dehydrogenase enzymes have been isolated and purified by affinity and ion-exchange chromatography. The reductase is capable of converting xylose, arabinose, and galactose to their respective sugar alcohols. Specific antibody has been produced to the reductase.

Demonstration of enzyme purifications (xylose reductase) will allow one to produce a selective protein probe to screen a Pachysolen clone bank for the presence of a gene/s responsible for xylose reductase synthesis. This

powerful technique allows one to determine whether the gene responsible has been transformed. (See F, A.1.)

Characterization of β -Glucosidase in Fermentative Yeast. This research is being conducted to develop a fast-growing, ethanol-tolerant hybrid yeast that has the capability to hydrolyze cellulosic intermediates directly to ethanol. In developing cloning strategies for the transformation of the β -glucosidase gene, purification of the enzyme has been demonstrated in Candida wickerhamii.

The enzyme responsible for the conversion of cellodextrins (cellulosic intermediates) to glucose has been purified from Candida wickerhamii. The native molecular weight is 200,000, a glycoprotein and capable of complete hydrolysis of cellodextrins $G_6 \rightarrow G_2$ (in vitro).

Purification of this novel β -glucosidase is significant in terms of producing a selective antibody to be used as a probe for identifying this enzyme in transformed yeast cells. (See F, A.1.)

Completed Full-Term Feeding Trial with Young Cattle Using Cattle Waste-Grain Ferment. To reduce feeding costs by removing the soy protein component of feed and to reduce on-site pollution, a continuous solid-substrate fermentor was used to produce feed from cracked corn and animal waste during a full-term feeding trial of cattle. Young cattle (14/group) were fed for an 82-day period. Test diet was corn-waste ferment (waste equals 14% dry matter) amended with urea after fermentation to match nitrogen level in corn-soy diet of controls. During feeding trials, two test animals showed displaced abomasa. Diet was then changed to include 20% silage as compared with 15% silage used in standard diet. At the end of feeding trial dry matter intake was 19.8 pounds/day (control) vs. 16.6 pounds/day (test animals); daily gain 2.4 pounds/day (control) vs. 2.15 pounds/day (test) and feed/gain of 8.3 (control) vs. 7.7 (test). Using fermented product: (1) approximately 20% less grain is required to produce weight gains equivalent to animals on control diet; (2) soy protein is replaced by animal waste and urea; and (3) pollution is reduced by recycling 1/3 of the waste through the fermentor into feed. (See F, A.3.)

ARS Culture Collection. The international recognition accorded the ARS Culture Collection has been recently enhanced through its successful operation as an International Depository Authority under the Budapest Treaty which was negotiated to internationally recognize the deposit of microorganisms for patent purposes. The Collection has now further increased its value to ARS research programs through the accessioning of two important collections. The first, Selman A. Waksman's collection of actinomycetes, contains many industrially important strains as well as those necessary for taxonomic comparisons. The second collection represents toxigenic strains from the Fusarium Research Center and will prove important to our programs in mycotoxin research. (See F, B.2.)

Clarification of Species Relationships in the Genus Bacillus. Because the soil bacteria Bacillus amylolyticus, B. lautus, B. pabuli, B. psychrophilus, and B. validus were inadequately described, they have not been recognized as valid species. DNA relatedness determinations have demonstrated that each of these species is genetically distinct, and characterization studies have established that each is phenotypically unique by homogeneous. Such findings suggest strongly that the five aforementioned species are valid taxa, and also demonstrate the effectiveness of the DNA/DNA reassociation technique for studying the genetic relationship of microorganisms. In addition, two groups in this study, which were originally misidentified, were found to produce alginase, an enzyme not usually observed in gram-positive bacteria. This research shows again that taxonomic studies and other research often are mutually beneficial: the taxonomy provided the correct identification of the enzyme-producing organisms, and the enzyme research uncovered a characteristic useful for differentiating some Bacillus species. (See F, B.2.)

Specific, Enzymic Cleavage of a Rhizobial Exopolysaccharide. Many strains of nitrogen-fixing bacteria that nodulate soybean plants form a surface carbohydrate polymer bearing an unusual sugar residue as regular side-branches. Soybeans contain a protein that specifically binds the unusual sugar. This cross-binding might lead to specific, competitive nodulations. Validation of this idea requires precise knowledge of: carbohydrate structures involved; location of the protein on roots; and basis for cross-nodulation by bacteria associated with tropical legumes. Because small, binding fragments can help characterize such interactions, an enzyme was sought that specifically cleaves the carbohydrate polymer. A soil bacterium was isolated that excretes the desired enzyme, which splits the polymer into single repeat units of four linked sugars. This tetramer has the unusual sugar at one end. It binds to the protein and, when attached to an inert carrier, was used to fish out the binding protein from a crude seed extract. The purified protein will be used to elicit an antibody that can detect it in roots and other soybean tissues. Identically constituted carbohydrate polymers from some tropical bacteria that cross-nodulate soybeans also react with the protein. Two of these polymers were resistant to enzyme cleavage because they have a different linkage at the usual point of enzymic attack. (See F, C.1.)

Study of Deoxynivalenol (Vomitoxin) in 1982 Soft Red Winter Wheat and in Flour Made from 1982 Hard Red Winter Wheat. An emergency study of deoxynivalenol (DON) in soft red winter (SRW) wheat was made because Fusaria or scab damage was reported to be extensive in SRW wheat being harvested in 1982. DON is produced by Fusaria mold. Samples (44) were collected to be representative of the SRW wheat harvested that year. Of the 44 samples analyzed, only one had more than 1 ppm (1.04 ppm) DON. The suggested level of concern of the FDA for DON in wheat at harvest was 2 ppm. The level for DON in wheat accepted by the Canadian Health Protection Branch is 2 ppm. There were correlations between DON levels and the grading factors--total damaged kernels, total damage, and total scab damage.

Samples of flour prepared from 1982 hard red winter (HRW) wheat representative of five areas in Kansas and Nebraska where scabby wheat had been identified also were analyzed. Of the 120 samples analyzed, 74% had 1 ppm DON or less and would be acceptable if levels of concern suggested by the FDA were adopted. These levels would also meet the standards of the Canadian Health Protection Branch. The mean level of DON in flour samples was 0.76 ppm which could have been predicted by the mean level in wheat harvested in the same area in 1982--1.5 ppm. Milling studies have shown that flour has about one half the level of DON present in the original wheat. The results allayed fears that threatened orderly marketing of the 1982 crop worldwide. (See F, D.1.)

Development of Analytical Methods for Aflatoxin in Small Samples of Corn and Airborne Dusts from Corn. The hazard to farmers and elevator workers from mycotoxins in the dust from harvesting and conveying grain is of grave concern. No methods existed for measuring aflatoxin in samples of airborne dusts from corn collected on filters in personal air samplers. High performance thin layer chromatography (HPTLC) was used to measure aflatoxin in extracts purified by silica gel 60 (0.040-0.063 mm particles) chromatography. Recoveries were satisfactory. The limit of detection of aflatoxin B₁ was 0.01 ng on a HPTLC plate. The limit of detection of B₁ in a 0.1 g sample of corn was 0.5-1.0 ppb. The methods for determining aflatoxin in 1.0 and 0.1 g samples were applied to airborne dust samples generated from naturally contaminated corn. These methods are being applied in NIOS sponsored studies to assess the safety of agricultural workers. (See F, D.1.)

Cyclic Peptide Structure Determined. As part of a program aimed at developing a more complete profile of metabolic products of fungi from grains, a complex with the unique property of causing cells to swell was isolated from species of Fusarium. In a cooperative project with the Massachusetts Institute of Technology, the structure of this material has been established as a mixture of three cyclic peptides. The amino acid sequence common to all is threonine-alanine-alanine-glutamine-tyrosine; they differ in the nature of the sixth amino acid (either leucine, isoleucine, or valine). The two ends of the hexapeptide are linked to form a cyclic structure by combination with a hydroxy fatty acid. This complex is highly inhibitory to growth of the fungi Penicillium and Aspergillus by causing their conidia to swell and then collapse. The material apparently acts by impairing the membrane properties or cell wall structures essential to all living cells. (See F, E.1.)

Detoxification of Epoxytrichothecenes. Feed refusal is one of the characteristic responses of swine confronted by diets that contain the epoxytrichothecene class of mycotoxins. In order to determine which structural features are necessary for refusal activity, six derivatives of T-2 toxin were prepared and evaluated using a mouse drinking-water bioassay. A reaction sequence of potential utility for decontamination of feeds or other materials containing T-2 toxin was developed; it consists of treatment with ammonium hydroxide followed by hot water. The major products of this detoxification reaction do not have refusal activity. This hydration approach may be the basis for a detoxification process, because no hazardous or difficult to

remove reagents need to be added to destroy the portion of the epoxytrichothecene molecule responsible for refusal activity. (See F, E.1.)

Characterization of Oat Oil Constituents Attractive to the Sawtoothed Grain Beetle (*Oryzaephilus surinamensis*). Oat oil has been fractionated extensively and analyzed in order to pinpoint compounds (or classes of compounds) attractive to *Oryzaephilus surinamensis* (sawtoothed grain beetles), a major pest of stored grain products. The most attractive volatile compounds identified were two aldehydes, (E)-2-nonenal and (E,E)-2,4-nonadienal; several other aldehydes present were found to be attractive to this beetle, but less intensely so. Dimethyl esters of succinic, glutaric, and adipic acid also are attractive. Triglycerides of oat oil also stimulate aggregation of *O. surinamensis*, especially those which incorporate only such common fatty acids as palmitic, oleic, and linoleic acids. On the other hand, triglycerides containing oxidized fatty acids--those with epoxy or hydroxydiene groupings--induced a distinct avoidance response. It is expected that these findings will facilitate development of trapping techniques for the sawtoothed grain beetle. [See Horticultural and Special Crops Laboratory (HSC), A.3.]

Maytansinoids from Trewia nudiflora. A new group of maytansinoid compounds, different from those known previously, has been isolated from seed of *Trewia nudiflora* (Euphorbiaceae) and characterized structurally. Like some previously known maytansinoids, members of this new group exhibit antitumor properties. In addition, they are found to affect significantly various pest insects (European corn borer, striped cucumber beetle, codling moth) in ways that indicate potential use of these compounds as natural pest control agents. (See HSC, A.3.)

Benzyl Isothiocyanate Inhibits Germination of Velvetleaf. It was discovered that benzyl isothiocyanate (BITC) isolated from papaya seed inhibits the germination of velvetleaf seed without inhibiting the germination of corn. The methodologies included the extraction, isolation, and characterization of BITC which was found to inhibit germination in an ongoing project which examines extracts from uncultivated seed for germination inhibitors. The bioassay was developed specifically for those extracts or compounds which affect velvetleaf. The discovery of BITC as a specific germination inhibitor provides agriculture and industry a structure which could be the basis for a future comprehensive herbicide for velvetleaf. (See HSC, B.1.)

Control Mechanisms for Plant Lipid Synthesis. Acyl carrier protein (ACP) is a key cofactor in plant lipid biosynthesis. The protein is essential for synthesis of both cell membranes and the storage triglycerides which account for more than 1/3 of the carbon stored in soybean seeds. Because of its central role in lipid metabolism, ACP has been chosen to study the control of fatty acid production in the developing soybean seed. To carry out these studies, two new assays for ACP were developed. The first measures active ACP using an enzyme isolated from *Escherichia coli*. The second is a radio-immunoassay which uses antibodies specific for ACP to measure total ACP in the plant tissue. These two assays have been used to measure levels of ACP

at different stages of soybean development and the data have led to important insights into how lipid synthesis is controlled. It has been discovered that both ACP activity and total ACP protein continue to increase in the soybean cells even after cell division has ceased and that there is a close correlation between in vivo fatty acid accumulation and the level of ACP. These results indicate that the level of fatty acid biosynthetic proteins such as ACP is a major determinant of the capacity of the seed to produce storage lipids. The results also provide a foundation for exploring the regulation of lipid production at the level of gene expression. (See HSC, B.2.)

Identification of Ergot Cyclol Alkaloids in Crude Extracts by Tandem Mass Spectrometry. Ergot alkaloids which are produced by fungi can cause toxic syndromes ("ergotism") in domestic livestock grazed on pastures or grains that contain sufficient levels of the alkaloids. Mass spectrometry/mass spectrometry (MS/MS), a technique to identify specific compounds in complex mixtures of organic compounds, has been successfully applied to the detection of the ergot peptide alkaloids. The method is sensitive and rapid. One solvent extraction with chloroform and ammonium hydroxide of the defatted grass, seed or feed sample, is made. An aliquot equivalent to 1 mg of sample is then analyzed by MS/MS. Using MS/MS, all the ergot peptide alkaloids can be individually detected at levels below 100 parts-per-billion directly from this simple extract of the sample. This procedure is substantially more rapid and requires less sample cleanup and purification than previous methods. (See HSC, B.4.)

Identification and Quantitation of Aflatoxin B₁ in Corn by Tandem Mass Spectrometry. Aflatoxin B₁ is a potent carcinogen formed under some conditions by certain fungi growing on corn. The presence of the toxin is a potential hazard to both animals and humans. Now, aflatoxin B₁ can be identified and quantitated at levels below 10 parts-per-billion in corn using mass spectrometry/mass spectrometry (MS/MS). The analysis can be made after a simple chloroform/water (80/20) extract of the ground corn sample without any sample cleanup. This rapid procedure allows mass spectral identification of aflatoxin B₁ in the corn matrix without needing the rigorous and hard-to-reproduce cleanup by preparative thin-layer chromatography used in the previously published mass spectral identification procedure for aflatoxin B₁. (See HSC, B.4.)

Absolute Evaluation of Physical Properties of Food and Structure-Property Relations. It has been possible to obtain basic data on the physical properties of foodstuffs which could be interpreted in fundamental terms. It was shown, for example, that for very viscous dispersions of swollen starch granules, properties depended only on the volume occupied by the swollen granules. For more solid-like materials such as starch gels, starch-hydrocolloid gels and protein gels, different measurement techniques including simple shear and torsion as well as dynamic methods were modified and developed; with these methods, it proved possible to relate the results from these different experimental techniques in fundamental terms using theories available from other fields in material and polymer science. The development of basic

measurements of food properties is an essential part of efforts to understand the complex interactions in food systems needed, for example, to foster increased use of vegetable protein in food. In addition, there is current concern about the precision, accuracy and reliability of published literature data on foodstuffs as shown by the recent efforts by European Economic Community through the "COST 90 Subcommittee on Rheological Properties of Foodstuffs" to improve the quality of physical property data on foodstuffs. (See HSC, C.1.)

Discovery in Chenopodium quinoa of the First Naturally Occurring Trisubstituted Saponin. Chenopodium quinoa is suitable for cultivation in semi-arid regions, and its seeds are a source of highly nutritious protein, but they contain bitter-tasting toxins that limit their use in food. Three advances were made in the process of isolating and characterizing these bitter toxins, the work being carried out in cooperative studies with Purdue University. First, it was shown that the toxins were mixtures of saponins which in turn are sugar derivatives of sapogenins. Second, the sapogenins were shown to consist only of oleanolic acid and hederogenin. Finally, a new class of compounds was discovered, termed tridesmoisides, which are saponins with three separate sugar residues attached to a sapogenin backbone. Such structural information is critical in tracing the way in which these undesirable compounds are formed in the plant as a first step in their elimination through genetic modification. Such plant improvement should permit wider use of the protein potential of quinoa in human food. (See HSC, D.1.)

Control of Photosystem Levels in Soybeans. Prospects for manipulating energy captured in photosynthesis improve continually as knowledge accumulates on light-gathering and energy-transferring structures in plants. Concepts of photosystem organization and operation suggest possibilities for preferential development of alternative pathways for energy conversion in photosynthesis and current research is defining such control at the molecular level. In soybeans the amounts of photosystems known to operate in green plants (PSI and PSII) have been shown to vary with degree of maturation, genotype, and light quality. Immature soybeans, chlorophyll-deficient genotypes, and plants grown under far-red light all have higher ratios of PSII/I than appropriate controls. Pigment changes during plant senescence also lead to enrichment of PSII, the system that produces oxygen and is the major source of chemical energy from photosynthesis. These studies demonstrate that, contrary to earlier theories, plants need not contain equal amounts of both photosystems. They also identify experimental conditions that separate factors controlling the preferential development of one photosystem versus the other. This accomplishment simplifies the remaining task of relating specific protein constituents to specific genetic messages and events that trigger their expression during plant development. (See HSC, D.2.)

Herbicide-Cofactor Competition in Photosynthesis. Enhanced herbicide resistance in crops and the ability to deal with resistance in weeds are expected to benefit agricultural efficiency. Both require knowledge of herbicide action. Among herbicides that disrupt growth by interfering with

photosynthesis, atrazine is known to inhibit energy flow by binding to a protein component of photosystem II (PSII). Reports linking structural change in this protein with atrazine resistance suggest a constitutive basis for resistance. Current research is defining functional conditions that must also be met to maximize photosynthesis in resistant plants. In the dark, atrazine binds to chloroplasts easily and interferes with the normal function of an electron acceptor, B, that conducts energy from PSII. In light, with B in a different functional state (reduced), atrazine binds poorly; i.e., the association of B with PSII varies considerably as it is alternatively reduced and oxidized during normal photosynthesis. If atrazine and B bind competitively, as data suggest, the development of atrazine-resistant plants could require special measures to maintain or improve energy transfer through B or similar electron acceptors. Such insight provides a specific subcellular objective for plant improvement. (See HSC, D.2.)

Soybean Calmodulin Stimulates Protein Phosphorylation in Photosynthesis. Soybean seeds and especially soybean hypocotyls yield a calcium-binding protein that stimulates phosphodiesterase activity. In this and in other ways it is like calmodulin, a known calcium-activated regulator protein that potentiates protein phosphorylations and thereby controls the activities of enzymes and other proteins and, thus, various cellular processes. This soy protein contains fewer methionine residues per molecule than does bovine calmodulin, which may account for subtle differences in specific activity and physical properties, but otherwise it should be considered a new addition to a growing group of non-enzymic metalloprotein regulators from plants. Both the soy protein and bovine calmodulin increase phosphorus incorporation into chloroplast proteins in a manner that is light- and calcium-dependent and calmodulin-dose responsive. The protein thus potentiates phosphorylation of proteins of the same molecular weight as those already known to be phosphorylated during photosynthesis. This implication of calmodulin in photosynthesis may eventually explain divalent cation effects on the distribution of absorbed radiant energy in plants. It identifies a specific protein involvement that, until now, could only be inferred. (See HSC, D.3.)

New Parameter Relates Triglyceride Structure to Behavior. Triglyceride molecules can assume a multitude of spatial configurations depending on treatment. This conditions imparts advantageous and disadvantageous properties to triglyceride-rich materials whether they be vegetable-based coatings, processed foods, or bioactive membranes. Efforts to optimize beneficial behavior and minimize the disadvantages in such materials are frustrated by poor understanding of lipid properties. New probes of this problem are allowed by improved instrumentation and computers; the emerging importance of biomembrane technology provides justification. Thus, a quantitative relationship noted earlier in computer modeling hydrocarbons for stabilities and structure is acquiring new significance. These studies discovered that a particular solid form is stable if the middle of the hydrocarbon chain contributes more than 60 percent of the molecule's stabilizing interaction energy. Current research shows that this relationship

also holds for saturated triglycerides. Major changes in their thermal properties occur when mid-chains contribute more than 60 percent of the total interaction. Such insight provides a valuable conceptual tool with which to pursue fundamental knowledge of more complex lipid structures and their interactions with non-lipids. (See HSC, D.4.)

Solid Lipid Transformation Defined. Two unsaturated fatty acid esters examined fortuitously in studies on lipid physical properties exhibit thermal behavior not detected previously in shorter- or longer-chain esters or other lipids. This chance discovery simultaneously provides insight into double bond position effects on properties and new concepts and methods with which to investigate factors that control polymorphic transformations in solid lipids. The work suggests that when molten lipids are cooled and solidified, specific crystal structure transformations initiate at temperatures that are characteristic for a given structure and well below the material's freezing point. These transformations proceed via first-order kinetics. Furthermore, depending on how thoroughly the solid is cooled, the path from melt to solid and back to melt may not be entirely reversible, and critical responses to conditions along the way determine the actual course of such a cycle. This concept is especially valuable in interpreting temperature effects on membrane processes. (See HSC, D.4.)

Supercritical Carbon Dioxide Extraction of Soybean Flakes. Hexane-defatted soybean flours have grassy-beany bitter flavors which inhibit their acceptability for human food uses. Full-fat soybean flakes were extracted with supercritical carbon dioxide (SC-CO₂) at 5000-12,500 psi, 50-100°C, and moisture levels 6-15%. Temperature, pressure, moisture, flow rates and time of extraction were all related. Conditions can be selected to produce defatted soybean meals with excellent protein solubility and flavor scores >6.5 on a scale of 1-10 (where 1 = strong and 10 = bland). The grassy-bean, bitter flavors of hexane-defatted soybean flours are minimally detectable in optimally SC-CO₂-extracted materials. Defatted soybean flakes prepared by SC-CO₂ extraction were further processed into improved protein products. The process should have potential in the food industry for the extraction of vegetable oils to yield proteins of superior flavor and physical qualities that make them especially promising as ingredients in food. [See Oilseed Crops Laboratory (OC), C.1.]

Biological Interactions of Lipid Oxidation Products with DNA. To clarify the biological effects of fat rancidity, the interactions of a wide variety of lipid oxidation products with DNA were investigated by determining the fluorescence formed in the presence of metals and reducing agents. The effect of malonaldehyde was also investigated because it has been generally considered among the most important lipid secondary oxidation products responsible for the formation of fluorescence with DNA. At low concentrations, hydroperoxides and secondary oxidation products from methyl linoleate and linolenate formed various degrees of fluorescence only in the presence of metals and ascorbic acid. In contrast, malonaldehyde produced very little fluorescence under our conditions. In our model reaction system we showed that DNA forms fluorescence products by the breakdown of lipid oxidation

materials other than malonaldehyde. Therefore, the biological importance of malonaldehyde may have been greatly exaggerated in the literature. (See OC, D.1.)

Flavor and Cooking Performance of Hydrogenated Soybean Oil. Continuous slurry hydrogenation was investigated to improve the flavor stability and cooking performance of soybean oil and to reduce serious economic losses reported by the military. New sensory methodology was developed to evaluate cooking oils. Room odor evaluations were carried out by heating oils to 190°C after frying bread, and taste panel evaluations were conducted on the bread cubes obtained after heating and intermittent frying. Soybean oils partially hydrogenated with copper and nickel catalysts to lower the amount of linolenate showed better frying performance than the unhydrogenated control oil. The most highly hydrogenated oils were scored poorly by our panels because of their strong hydrogenated-paraffin odors. Analyses of flavor volatile compounds by capillary gas chromatography showed that all hydrogenated oils after prolonged heating and bread frying produced less volatiles than the unhydrogenated control oil. In the presence of methyl silicone, tertiary butyl hydroquinone and citric acid, all odor scores were improved and volatile formation was decreased showing a synergistic effect. Therefore, better cooking performance and more economic advantages are achieved by using partially hydrogenated soybean oil with additives than soybean oil hydrogenated to remove linolenic acid completely. (See OC, D.2.)

Isolation of Trypsin Inhibitor (TI) Concentrate from Soybeans. The successful development of a pilot-plant process for extracting trypsin inhibitor (TI) from soy flour provides the means to make sufficient quantities of material to support chronic lifetime (2 year) feeding studies with rats. The TI fraction was separated from the non-TI protein at ambient temperatures by a combination of salting-in and isoelectric precipitation. Purification of the TI concentrate was accomplished by applying ultrafiltration techniques and the final product was freeze-dried. The final freeze-dried TI concentrates had an average purity of 35% TI. This is the first time that purified TI has been available in quantities needed to study the effect of the inhibitor in the digestive process and as a potentiator of pancreatic degeneration. (See OC, E.2.)

Storage Stability of Soybean Oil-Based Salad Dressings. To resolve important storage problems in procurement for the military, the flavor stability of salad dressings was investigated to determine the effect of hydrogenation of the oil, additives, and storage conditions. Flavor quality tests were developed and correlated with gas chromatographic analyses of volatile flavor compounds in oils separated from the emulsion dressings. Hydrogenation of soybean oil with copper and nickel catalysts effectively increased the storage stability of salad dressings at 70°F but not at 90°F. The use of BHA as an antioxidant in the oil or EDTA as a metal inactivator in the starch base as well as nitrogen packaging were effective in prolonging the storage stability of salad dressings made with unhydrogenated soybean oil. No synergistic effect was evident from hydrogenation combined with additives

or nitrogen packaging. Therefore, the use of antioxidants, EDTA or nitrogen packaging may provide economic substitutes for hydrogenation of soybean oil used in salad dressings.

BIOMATERIALS CONVERSION LABORATORY

W. M. Doane, Chief

A. Biochemical Engineering Research

R. J. Bothast, Research Leader

1. New and Improved Biotechnologies for the Fermentative Production of Chemicals from Biomass (R. J. Bothast)

Bacterial cultures from the ARS Culture Collection were screened for their ability to use glucose, xylose, glycerol, and a mixture of the three carbon sources. Conversion of glycerol to beta-hydroxypropionaldehyde was optimized with Klebsiella pneumoniae. Yield was improved from 13.1 g/L to 16.0 g/L.

Continuous culture selection of rapidly growing strains of Pachysolen tannophilus was accomplished by subjecting a mutagenized cell population growing aerobically on D-xylose to high dilution rates over 6 weeks. Mutant isolates were identified that have growth rates approaching fast-growing strains of Saccharomyces.

Publications:

SLININGER, P.J., BOTHAST, R.J., and SMILEY, K.L. 1983. Production of 3-Hydroxypropionaldehyde from glycerol. Appl. Environ. Microbiol. 46:62-67.

BOLEN, P.L. and SLININGER, P.J. Continuous culture selection of mutant strains of Pachysolen tannophilus capable of rapid aerobic growth on D-xylose. Dev. Ind. Microbiol. In press.

2. Hydrolysis of Hemicelluloses by Enzymes from Anaerobic Bacteria (Cooperative Agreement - Virginia Polytechnic Institute and State University)

The hemicellulosic components of wheat straw were partially characterized, and the ability of selected anaerobic bacteria from the human colon to ferment wheat straw, its hemicellulose fractions, and commercial xylan was evaluated. Of 12 strains of Bacteroides sp. tested, none fermented wheat straw. Six of seven strains of B. ovatus fermented crude hemicellulose and hemicelluloses A and B. Five strains of B. vulgatus, B. uniformis, and B. thetaiotaomicron did not ferment hemicelluloses. Detailed studies with Strain B4-11 of B. ovatus showed that about 30.3% of crude hemicellulose and 8.8% of heteroxylan were hydrolyzed with an intracellular enzyme fraction. Xylose was the major sugar released from crude hemicellulose, while nearly equal amounts of xylose and arabinose were released from heteroxylan. β -1,4-xylosidase was the predominant enzyme in the intracellular enzyme fraction.

Publications:

REDDY, N. R., PALMER, J. K., PIERSON, M. D., and BOTHAST, R. J. 1983. Wheat straw hemicelluloses: Composition and fermentation by human colon Bacteroides. J. Agric. Food Chem. 31:1308-1313.

REDDY, N. R., PALMER, J. K., and PIERSON, M. D. Hydrolysis of wheat straw hemicelluloses and heteroxylan (larchwood) by human colon Bacteroides ovatus Strain B4-11 enzymes. J. Agric. Food Chem. (in press).

B. Chemical Engineering Research

G. E. Hamerstrand, Research Leader

1. Recovery of Rubber from Guayule and Related Whole Plant Materials (G. E. Hamerstrand)

A gravimetric method developed at NRRC in cooperation with the U.S. Water Conservation Laboratory in Phoenix, AZ was selected as the Standard Method of the Guayule Rubber Society for determining the resin and rubber content in guayule. A computer assisted near infrared spectrophotometer has been successfully programmed to quantitatively measure the rubber and resin, as well as the moisture content, of ground, whole-plant material. Analysis time has been reduced from the approximately 1 hour per sample required in the gravimetric procedure to less than 1 min in the instrumental method allowing rapid screening of the large number of samples being generated in agronomic studies. Rubber quality, as measured by molecular weight (M.W.) was found to be very sensitive to storage conditions. In ground samples stored at temperatures typical of those encountered in the Southwest (100+°F) the M.W. declined as much as 50% in a 24 hour period. Freezing the samples prevented appreciable decreases in M.W. during storage. Solvent to plant material ratios, feed rates, process temperatures, and contact times were evaluated in pilot-trials in conventional counter-current oilseed extraction equipment for the continuous extraction of both the resin and rubber components from guayule and milkweed with a dual solvent (acetone then hexane) system. Preliminary trials with a single solvent system (alcohol-hexane azeotrope) have shown that both the rubber and resin can be successfully removed from whole plant material in a single pass through the continuous extractor.

Publications:

BLACK, L.T., HAMERSTRAND, G.E., NAKAYAMA, F.S., and RIANIK, B.A. 1983. Gravimetric analysis for determining the resin and rubber content of guayule. Rubber Chem. Technol, Vol. 56:367-371.

HAMERSTRAND, G.E. and MONTGOMERY, R.R. Pilot-scale guayule processing using countercurrent solvent extraction equipment. Rubber Chem. Technol. In press.

C. Field Crops Post-Harvest Research

R. A. Anderson, Research Leader

1. Trickle Sulfur Dioxide Treatment for On-Farm Drying of Corn at Ambient Temperatures (Cooperative Agreement - Purdue University)

Field experiments on preservation of high-moisture corn with sulfur dioxide (SO₂) while drying with ambient air have demonstrated the efficacy of SO₂ for controlling fungal growth. Best method found for adding SO₂ to corn was by introducing gas at top of bin of corn and pulling it down through grain by reversing fan. In this system, SO₂ was at its highest level in top of bin, where the corn is dried last. Introduction of SO₂ at bottom of bin was satisfactory, but slightly less effective. Metering gas onto entrained corn in a loading auger with a one-time application was unsuccessful in inhibiting mold development.

Publication:

ECKHOFF, S.R., TUIITE, J., FOSTER, G.H., ANDERSON, R.A., and OKOS, M.R. Sulfur dioxide as a mycocidal adjunct for low temperature grain drying. Trans. ASAE (accepted for publication).

D. Hydrocarbon Plants and Biomass Research

M. E. Carr, Research Leader

1. Hydrocarbon-Producing Plants as Potential Multi-Use Crops (M. E. Carr)

Whole-plant specimens of 625 species were collected. Of these, 350 were evaluated for their potential as energy-producing crops. Oil and hydrocarbon from 85 selected species were partially characterized for lipid classes, for yields of fatty acids and unsaponifiable matter, for rubber, gutta, and/or waxes in hydrocarbon by IR and ¹³C NMR, and for MW and MW distribution of rubber and gutta by GPC. Of the 350 species 35 were identified for future study. Promising species yielded as much as 10% oil (Pinus albicaulis), 26% polyphenol (Acer ginnala), 2.3% hydrocarbon (Pittosporum tobira), and 23% apparent protein (Verbesina encelioides), on a dry, whole-plant basis. Two new sources of gutta were discovered (Garrrya flavescens and G. wrightii). A GPC technique was developed to quantitate rubber. For a heritability study, 78 leaf samples of Asclepia syriaca were analyzed for oil, polyphenol, hydrocarbon, and protein. For 12 USDA plant scientists, 625 sugar crop samples (1982 harvest) were analyzed for sugars and 580 samples (1980 harvest) for lignin and cellulose. Five species in 25-pound quantities were collected for gasification and biomass quality study for a cooperating university. Hydropulping studies of oil- and hydrocarbon-producing plants (e.g., milkweed, guayule, and sunflower) after solvent extraction were conducted to enhance their multipurpose usefulness.

Publications:

CARLSON, K. D., CUNNINGHAM, R. L., and HERMAN, A. I. Sweet sorghum grown on sludge-amended stripmine soil: A preliminary look at yields, composition, and ethanol production. Trans. Ill. State Acad. Sci., in press.

CARLSON, K. D., CARR, M. E., CUNNINGHAM, R. L., BAGBY, M. O., and PALMER, D. M. Lignin analyses on sweet sorghum samples. Trans. Ill. State Acad. Sci., in press.

2. Model for Determining Feasibility of Potential Energy Crops (Cooperative Agreement - Purdue University)

Employing dynamic approaches, analysis procedures were developed based on crop energy input/output data, growth and development relationships, storage losses, and processing methods to allow comparison of "energy crops" to each other and to conventional crops. Using sweet sorghum as an example crop, a model was developed for its yield under non-irrigated eastern corn belt conditions with variable planting (May 3 to June 20) and harvesting (July 12 to October 31) dates by modifying a grain sorghum model using literature inputs and one-year field trial data (1982) at West Lafayette, IN. The multi-stage linear program selected sweet sorghum as a major crop at \$75/Tonne (dry weight) in a scenario for optimal cropping plan (considering factors such as: yield vs. data for harvest and planting, machinery sizes, and energy input) including options to produce competing crops of corn, soybeans, winter wheat, and double-cropped soybeans following winter wheat. The diverse crop scheme provides a significant advantage over more limited schemes by providing for extended planting and harvesting periods, smoother demand for machinery, and lower machinery fixed costs.

Publication:

BENDER, D. A., VANDERLIP, R. L., SMITH, G. A., BAGBY, M. O., and PEART, R. M. 1983. Simulating the growth and development of sweet sorghum. ASAE Paper No. 83-3022, American Society of Agricultural Engineers, St. Joseph, MI.

E. Physical Chemistry Research

W. M. Doane, Acting Research Leader

1. Plant Component Separation and Physical Characterization (T. P. Abbott)

Rates and extent of delignification of kenaf by 12 species of Cyathus fungi were determined. Several aspects of the chemical mechanism of alkaline H₂O₂ delignification of lignocellulosics were elucidated, and the efficient conversions of cellulose to glucose and cellulose to ethanol were demonstrated. Lignin is oxidized by the peroxide to short

chain mono and dibasic acids and to aromatic acids. Cell membrane vesicles from the lignin-degrading fungus Phanerochaete chrysosporium were isolated and a trans membrane glucose/H⁺ transport system was characterized. Extracellular H₂O₂ production by P. chrysosporium associated with lignin biodegradation was found to be induced by the presence of lignocellulosics in the culture medium. An active fatty acyl-CoA oxidase activity was discovered in P. chrysosporium mycelia which may represent the metabolic source of the excreted H₂O₂. Sweet sorghum stalks treated with propionic acid and stored under anaerobic conditions showed no loss in sugar content during the first 100 days. Wheat straw in the presence of various chemicals was rapidly modified in single- and twin-screw extruders to yield water extractable lignin and pentosan components and a cellulose residue highly accessible to cellulase. Treatment of wheat straw with dilute refluxing trifluoroacetic acid (TFA) selectively hydrolyzes the hemicellulose component and produces a solution of xylose that can be directly fermented to ethanol with Pachysolen tannophilus after removal of volatile TFA by evaporation.

Publications:

ABBOTT, T.P. and JAMES, C. 1983. Products of wheat straw biodegradation by Cyathus stercoreus. ACS Symposium Series 214, Am. Chem. Soc. 267-284.

ABBOTT, T.P. and JAMES, C. Kenaf lignin ¹⁴C-labeling and analysis. Tappi. In press.

BELL, D.H., PATTERSON, L.K. and GOULD, J.M. 1983. Transmembrane pH gradients and functional heterogeneity in reconstituted vesicle systems. Biochim. Biophys. Acta 725:368-375.

CARR, M.E. and DOANE, W.M. Modification of wheat straw in a high-shear mixer. Biotechnol. Bioeng. In press.

GOULD, J.M. 1983. Probing the structure and dynamics of lignin in situ. What's New in Plant Physiology 14(2):5-8.

GOULD, J.M. Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. Biotechnol. Bioeng. In press.

GOULD, J.M. High efficiency ethanol production from lignocellulosic residues pretreated with alkaline H₂O₂. Biotechnol. Bioeng. In press.

GREENE, R.V. and GOULD, J.M. Electrogenic symport of glucose and protons in membrane vesicles of Phanerochaete chrysosporium. Arch Biochim. Biophys. In press.

GREENE, R.V. and GOULD, J.M. 1983. Substrate induced H₂O₂ production by mycelia from the lignin degrading fungus Phanerochaete chrysosporium. Biochem. Biophys. Res. Commun. 117:275-281.

GREENE, R.V. and GOULD, J.M. Fatty acyl Coenzyme-A oxidase activity and H₂O₂ production in Phanerochaete chrysosporium mycelia. Biochem. Biophys. Res. Commun. In press.

2. Improving Feed Value of Lignocellulosic Materials (Cooperative Agreement - University of Illinois)

Lignocellulosic crop residues such as wheat straw and corn stover were partially delignified by treatment with alkaline hydrogen peroxide and tested for in situ dry matter digestibility using fistulated cattle. In each case peroxide treatment increased digestibility of the residue to nearly 100% after 48 hours residence in the rumen. Metabolic feeding trials of peroxide-treated wheat straw were begun using lambs restrained in crates that allowed collection of urine and feces for subsequent analysis. Preliminary analysis of the data indicates that lambs fed a diet containing 70% treated wheat straw gained weight and utilized energy in feed as well as lambs fed a mainly corn diet. Lambs fed a diet containing 70% untreated wheat straw lost weight during the trial.

F. Renewable Resources Research

F. H. Otey, Research Leader

1. Basic Studies on Modification of Natural Polymers as Replacements for Petroleum-Derived Polymers (F. H. Otey)

Highly transparent films were prepared from compositions of up to 60% starch, various levels of poly(ethylene-co-acrylic acid) (EAA) and strong alkali. Preliminary diffusion studies revealed the films have promising semipermeable characteristics. Observed permeabilities of six solutes were determined for four separately prepared films using a rotating dialysis cell. After 1 hour exposure of 1.5% solute concentration (w/w), urea diffused through one film 7.6 times faster than glucose and 32 times faster than sucrose. Increasing starch levels or incorporating water-soluble compounds into the films significantly increased diffusion rates. Incorporating water-soluble, biodegradable plasticizers and low-density polyethylene into starch EAA films increased the rates of biodegradation without adversely affecting plastic properties. Modifying starch or guar with cationic groups did not improve their ability to stabilize soil particles.

Cellulose-graft-poly(acrylic acid) reacted with hexadecyltrimethylammonium bromide to yield copolymers that remove emulsified oil from water. Starch-graft-poly(methyl acrylate) heated at elevated pH levels produces, on extrusion, continuous ribbons having improved toughness and tensile strength.

Cellulose-graft-poly(methyl acrylate) produced continuous plastic extrudates that were more brittle than their starch and hemicellulose

counterparts; however, removal of a portion of the cellulose with enzyme gave a product with properties more similar to those of the other two polysaccharide graft copolymers.

Publications:

FANTA, G.F., ABBOTT, T.P., HERMAN, A.I., BURR, R.C., and DOANE, W.M. Hydrolysis of wheat straw hemicellulose with trifluoroacetic acid. Fermentation of xylose with Pachysolen tannophilus. Biotechnol. Bioeng., in press.

FANTA, G.F., BURR, R.C., and DOANE, W.M. 1983. Ceric-initiated polymerization onto polyacrylonitrile with carbohydrate end groups. Graft versus block copolymer formation. J. Polym. Sci.: Polym. Chem. Ed. 21:2095-2100.

FANTA, G.F., SWANSON, C.L., BURR, R.C., and DOANE, W.M. 1983. Graft copolymers of methyl acrylate onto bleached wood pulp: Preparation and extrudate properties. J. Appl. Polym. Sci. 28:3003-3009.

FANTA, G.F., SWANSON, C.L., BURR, R.C., and DOANE, W.M. 1983. Polysaccharide-g-polystyrene copolymers by persulfate initiation: Preparation and properties. J. Appl. Polym. Sci. 28:2455-2461.

MAHER, G.G. 1983. Alkali gelatinization of starches. Staerke 35:226-234.

MAHER, G.G. 1983. Alkali gelatinization of flours. Staerke 35:271-276.

OTEY, F.H. 1983. Starch-based plastics and related products for agriculture. Proceedings of the 17th annual meeting of the National Agricultural Plastics Congress, Manchester, New Hampshire, July 5-8. In press.

OTEY, F.H. and WESTHOFF, R.P. 1983. Starch-based films--preliminary diffusion evaluation. Ind. Eng. Chem. Prod. Res. Dev. In press.

PRYDE, E. H. and OTEY, F.H. 1983. Renewable raw materials for the polymer industry. Polym. Almanac. In press.

SWANSON, C.L., FANTA, G.F., FECHT, R.G., and BURR, R.C. 1983. Starch-g-poly(methyl acrylate)--Effects of graft level and molecular weight on tensile strength. Polymer Applications of Renewable-Resource Materials, C.E. Carraher, Jr. and L.H. Sperling, Eds., Plenum, p. 59-71.

WEAVER, M.O., OTEY, F.H., and DOANE, W.M. 1983. Effect of some anionic starches of the stability of soil particles in water. Staerke. In press.

2. Basic Studies on Biopolymers for Improving Safety of Pesticides (F. H. Otey)

Bacterial and fungal alpha-amylases were found to release starch encapsulated trifluralin under mild conditions. Encapsulation was done

by the xanthate, calcium, and borate procedures and encapsulated products were subjected to the action of the alpha-amylases at pH 6 to release active ingredient in the order of borate more than calcium more than xanthate. Release rates were increased by increasing amounts of enzyme or decreasing particle size, were unchanged by varying the trifluralin concentration in the particles, and were decreased by crosslinking or retrograding the starch matrix. Particles of encapsulated trifluralin finer than 35 mesh showed promise in wet spray mix applications, but more work is necessary to prevent the particles from settling. Samples of encapsulated pesticides and seeds coated with encapsulated herbicides were supplied to collaborators for field testing. Dr. M. Schreiber (ARS, Purdue U.) found that starch encapsulated trifluralin provided good weed control in soybeans without being incorporated into the soil. Starch-encapsulated thiocarbamate herbicide gave better weed control in corn than the nonencapsulated herbicide when soil incorporation was delayed for several days. When applied in the fall, starch-borate encapsulated trifluralin was not as effective as starch-xanthate encapsulated trifluralin, but both were more effective than a commercial formulation. When applied in the spring just prior to crop planting, the xanthate product was more effective than the borate one. Samples of encapsulated malathion were prepared and submitted to Dr. Wendell Burkholder (ARS, Madison, WI) for evaluation of insect control in stored grain.

Publications:

SHASHA, B.S. 1983. Encapsulation by entrapment within starch adduct matrix. U.S. Patent 4,382,813.

SHASHA, B.S., TRIMNELL, D. and OTEY, F.H. 1983. Starch-borate complexes for EPTC encapsulation. J. Appl. Polym. Sci. in press.

TRIMNELL, D. and SHASHA, B.S. 1983. Weed control via encapsulated herbicides. Abstracts of the 1983 Meeting of the Weed Science Society of America. St. Louis, Missouri, February 8-10, p. 105, Abstract 252, Poster Session (XIV).

WING, R.E. and OTEY, F.H. 1983. Determination of reaction variables for the starch xanthide encapsulation of pesticides. J. Polym. Sci., Polym. Chem. Ed. 21:121-140.

WING, R.E. and SHASHA, B.S. 1983. Encapsulation of organic chemicals within a starch matrix. J. Chem. Ed. 60:247-248.

CEREAL SCIENCE AND FOODS LABORATORY

G. E. Inglett, Chief

A. Biologically Active Substances Research

G. E. Inglett, Acting Research Leader

1. Molecular Structure of Maillard-Type Browning Reaction Products (H. B. Sinclair)

Melanoidins were prepared from reactions of glycine with glucose, fructose, hydroxymethylfurfural and glyoxal. Both Cu-binding and Ca-binding studies were conducted with these products, all of which behave like acidic forms of anionic polymers providing clues on the structure of melanoidins. Nonenzymic browning product, galactosylisomaltol, was transformed by a mixture of triethylamine and pyrrolidine into D-galactosan. Free amino acids and amino functional groups of proteins and peptides in dough could probably act on lactose in Maillard-type browning reaction to produce galactosylisomaltol; whereupon, galactosylisomaltol undergo further degradation on contact with hydroxide ions to form D-galactosan during the baking process. Synthesis of 6-O-methyl-D-glucose was perfected. A modified synthesis of earlier researchers consisted of five steps and when performed without isolation of intermediates, it gave pure crystalline 6-O-methyl-D-glucose in excellent yield. From the 6-O-methyl-D-glucose will be prepared Amadori compound. Reaction of 5,6-anhydro-2,4-O-benzylidene-1-O-p-tolylsulfonyl-D-glucitol with base did not follow the reaction pathway as reported in the literature which was anhydro ring opening and then tolylsulfonyl displacement. The actual sequence was proven as the inverse, tolylsulfonyl displacement and anhydro ring opening.

Publications:

GOODWIN, J. C. 1983. Isolation of 3-O- α -D-gluco- and 3,0- β -D-galactopyranosyloxy-2-furyl methyl ketones from nonenzymic browning of maltose and lactose with secondary amino acids. Carbohydr. Res. 115:281-287.

FISHER, B. E., SINCLAIR, H. B., and GOODWIN, J. C. 1983. Synthesis of a derivative of 1-deoxy-D-erythro-2,3-hexodiulose and its conversion into nonenzymic browning products. Carbohydr. Res. 116:209-215.

SINCLAIR, H. B. 1983. Reaction pathway in the base transformation of 2,4-O-benzylidene-1,6-di-O-p-tolylsulfonyl-D-glucitol into 1,3-anhydro-2,4-O-benzylidene-D-glucitol Carbohydr. Res. 113:321-325.

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SINCLAIR, H. B. 1983. Convenient preparation of 1,4-anhydro-D-mannitol. Carbohydr. Res. (Accept for pub).

VAN CLEVE, J. W. and INGLETT, G. E. Amadori compounds from monomethylglucoses. A practical synthesis of 6-O-methyl-D-glucose. Abstr. Papers Am. Chem. Soc. 187. (Accept for pub).

RENDLEMAN, J. A., JR., and INGLETT, G. E. Influence of cupric ion in the Maillard reaction of glucose with glycine. Abstr. Papers Am. Chem. Soc. (Accept for pub).

2. Basic Research on Metabolic Regulation of Plant Tissues (G. E. Inglett)

Seeds from bioregulator-treated normal and mutant plants were morphologically similar to seed from control plants even though seed production was drastically reduced in some treatments. White maize seed from plants treated with 4 levels of 1,1-dimethylpiperidinium chloride did not show any morphological differences. Field grown corn plants exogenously applied with 16 different bioregulators gave a broad range of corn ear and grain yields attributable to specific bioregulators used. Levels of zinc and copper in the corn grain harvested, that included all 16 bioregulators plus control treatments, were significantly higher than in the corn seed from where they originated. The change in amylase activity of corn during germination and early seedling growth stages was studied on one inbred variety, one hybrid variety of normal amylose content, and two high-amylose genotypes of this hybrid. Application of exogenous gibberellic acid (GA_3) increased amylase activity of the inbred and the normal amylose hybrid about 30%. Presence of calcium in the imbibing medium accelerated growth of the embryo in all the varieties but had no effect on starch consumption. Lipids from a particulate fraction isolated from corn endosperm was shown to be composed of mainly phospholipids, and monogalactosyl and digalactosyl diglycerides indicating that these organelles originated from plastids; however, the particulate had physical properties resembling microsomes.

Publications:

PLATTNER, R. D., GARDNER, H. W., and KLEIMAN, R. 1983. Chemical ionization mass spectrometry of fatty acids: The effect of functional groups on the CI spectra. J. Am. Oil Chem. Soc. 60:1298-1303.

GARCIA, W. J., CAVINS, J. F., INGLETT, G. E., HEAGLE, A. S., and KWOLEK, W. F. 1983. Quality of corn grain from plants exposed to chronic levels of ozone. Cereal Chem. 60:388-391.

GARDNER, H. W. 1983. Effects of lipid hydroperoxides on food components. In "Xenobiotics in Foods and Feeds," ACS Publication No. 234,

GARDNER, H. W., WEISLEDER, D., and NELSON, E. C. 1983. Acid catalysis of a linoleic acid hydroperoxide: Formation of epoxides by an intramolecular cyclization of the hydroperoxide group. J. Org. Chem. (in press)..

GARDNER, H. W. 1983. Flavors and bitter tastes from oxidation of lipids by enzymes. In "Flavor Chemistry of Fats and Oils," AOCS Publication (Accepted for publ.).

B. Cereal and Food Biochemistry Research

F. R. Dintzis, Research Leader

1. Enzymatic Conversion of Cellulose to Sugars for Alcohol Fermentations (F. R. Dintzis)

Initial studies on isolation and characterization of a "distruption factor" from the cellulase enzyme complex of Trichoderma reesei are completed. Alternative methods such as solvent extraction and use of HPLC show promise as routes to increase yields of this factor, which has been shown to be active upon certain plant cell wall structures as well as filter paper.

Publication:

GRIFFIN, H. L., DINTZIS, F. R., KRULL, L. H., and BAKER, F. L. 1983. A microfibril generating factor from the enzyme complex of Trichoderma reesei. Biotechnol. Bioeng. (Accepted for publ.)

2. Factors Influencing Bioavailability of Minerals in the Presence of Food Fiber (F. R. Dintzis)

Mineral content comparisons of some wheat brans, dry-milled corn brans, and soy hulls measured prior to and after passage through the human GI tract reveal that retrieved wheat bran tissues contain significantly more Ca; corn bran remnants contain significantly more Ca, Zn, Cu, and Fe, while soy hull remnants contain less Fe and more Zn. Recent data indicate the effects of phytate content of modest amounts of fiber sources added to realistic human diets correlate with dietary protein. Thus, 0.764 g/day of Ca are required on a low protein-low phytate diet while 0.669 g/day are required on a low protein-high phytate diet. High protein diets demonstrated no correlation between phytate content and Ca requirement. The NDF content of swine digesta was not significantly different from that of fecal matter, whether a basal diet or a wheat bran supplemented diet was fed. From these results we infer that minimum breakdown of dietary fiber sources, corn bran and wheat bran, in these diets, took place in the pig colon. Loss of uronic acids from plant tissues may be reduced by shortened heating times in dilute sulfuric acid and by avoidance of excess heavy metal cations when removing sulfate ion from the digest. New gas chromatography methods have been developed which permit the simultaneous quantitation of aldonic acids and alditols, or aldonic acids and aldoses, or uronic acids and aldoses.

Publications:

GRAF, E. 1983. Formation of [^3H , ^{32}P]phytic acid in germinating wheat. Anal. Biochem. 131, 351-355.

GRAF, E. 1983. Calcium binding to phytic acid. J. Agric. Food Chem., Vol. 31, No. 4, 851-855.

3. Comparison of Factors in Human and Swine Digestive Processes Affecting Dietary Fiber and Minerals (Cooperative Agreement - University of Kentucky)

Dry-milled corn bran fiber is inefficiently digested by growing pigs, and its level of dietary inclusion should be limited to 0-6% neutral detergent fiber content. However, the nutritional and economic value of corn bran fiber was substantially greater in pigs housed in a cold versus warm environment. Microbial activity in the cecum and colon of pigs is significantly influenced by both corn bran levels as a dietary fiber source and environmental temperatures. Estimates of volatile fatty acid production rates in the cecum and colon suggest that this increase in pigs fed corn bran could provide additional energy for pigs grown in a hypothermal environment. The result that corn bran is inefficiently digested by growing pigs is similar to our findings that corn bran is inefficiently digested by humans.

C. Cereal Proteins Research

J. A. Bietz, Research Leader

1. Method of Analysis to Facilitate Genetic Improvement of Cereal Grain Protein (J. A. Bietz)

Procedures were developed and optimized for extraction, derivitization, and reversed-phase high-performance liquid chromatography (RP-HPLC) of all wheat proteins, permitting rapid varietal identification. Chromosomal loci coding gliadin and glutenin polypeptides, including those related to quality, were determined through aneuploid analysis. Durum gliadins associated with quality were identified and isolated; a rapid RP-HPLC test was devised for early-generation screening, and antibodies were produced to test structural relationships. Ion-exchange and size-exclusion HPLC were also used to isolate wheat proteins and predict baking quality. Proteins associated with wheat's high-protein and high-lysine characters were identified. RP-HPLC was used to isolate gliadins and gliadin peptides having opioid-like activity in a competitive binding assay. Procedures were developed to analyze corn zeins by 2-dimensional electrophoresis and RP-HPLC; genotypes and proteins linked to high-lysine genes were identified. Novel computer programs can identify components of hybrids, mixtures, and F2 populations. Differences between zeins and alcohol-soluble corn glutelin subunits were also observed, and polypeptides have been isolated for subsequent studies, which show that RP-HPLC

complements other methods. An N-terminal sequence was determined for a methionine-rich corn glutelin. Kafirins and alcohol-soluble sorghum glutelins were extracted and analyzed; their coding genes are close to high-lysine genes, and may indicate genotypic relationships.

Publications:

BIETZ, J. A. 1983. Separation of cereal proteins by reversed-phase high-performance liquid chromatography. *J. Chromatogr.* 255:219-238.

BIETZ, J. A. Analysis of wheat gluten proteins by high-performance liquid chromatography, Part I. *Bakers Digest* 58(2). (Accept for pub).

BIETZ, J. A., BURNOUF, T., COBB, L. A., and WALL, J. S. Wheat varietal identification and genetic analysis by reversed-phase high-performance liquid chromatography. *Cereal Chem.* in press.

BURNOUF, T. and BIETZ, J. A. Reversed-phase high-performance liquid chromatography of durum wheat gliadins: Relationships to pasta cooking quality. *J. Cereal Sci.*, in press.

BURNOUF, T., BIETZ, J. A., COBB, L. A., and WALL, J. S. 1983. High-performance liquid chromatography of gliadins from bread wheat varieties grown in France. *C.R. Acad. Sci. Paris* 297, III:377-382.

LANDRY, J., PAULIS, J. W., and FEY, D. A. 1983. Relationship between alcohol-soluble proteins extracted from maize endosperm by different methods. *J. Agric. Food Chem.* 31(6): 1317-1329.

WALL, J. S., FEY, D. A., PAULIS, J. W., and LANDRY, J. Improved 2-dimensional electrophoretic separation of zein proteins: Application to study of zein inheritance in corn genotypes. *Cereal Chem.*, in press.

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BIETZ, J. A., and SHARMA, G. C. 1983. Differences in endosperm proteins between yellow berry and normal triticales. *Crop Science* 23:704-708.

SHARMA, G. C., PAUL, A. D., and BIETZ, J. A. 1983. Nitrogen fertilization effects and anatomical, protein, and amino acid characteristics of yellow berry in Triticale. *Crop Science* 23:699-703.

WU, Y. V. 1983. Effect of germination on oats and oat protein. *Cereal Chem.* 60:418-420.

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BIETZ, J. A. 1983. Differences in selectivity of large-pore reversed-phase high-performance liquid chromatography columns for analysis of cereal proteins. Cereal Foods World 28(9): 555 (Abstract).

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BIETZ, J. A. 1983. HPLC: How Proteins Look in Cereals. Cereal Foods World 28(9):553 (Abstract).

LANDRY, J., PAULIS, J. W., and WALL, J. S. 1983. Isolation and characterization of M_r 22,000 subunits from zein. Cereal Foods World 28(9):557 (Abstract).

HUEBNER, F. R. and WALL, J. S. 1983. Variations in percent of protein fractions in high- and low-lysine wheats. Cereal Foods World 28(9):558 (Abstract).

2. Energy-Saving Methods for Recovery of Usable Protein from Alcohol or Methane Fermentation Media (J. S. Wall)

Residues from fermentation of hard and soft wheats and wheat flours were fractionated into distillers' grains, centrifuged solids, and distillers' solubles. Solid fractions were enriched in protein and lysine, but had reduced breadmaking properties. Soluble fractions from corn steep water and sorghum and wheat stillage, processed by ultrafiltration and reverse osmosis, yielded permeates low in nitrogen and dissolved solids. Blended foods were formulated containing corn meal, soy flour, nonfat dry milk, and high-protein corn distillers' dried grains (CDG). Reduced flavor acceptability was noted for these products, but improvement was achieved through extraction. Fiber content of CDG blends was reduced by dry milling and sieving. These studies suggest that products containing up to 10% CDG will be acceptable in blended foods. Studies of solubility and of sulfur amino acids in proteins from corn gluten meal (CGM), a product of wet milling; indicated that disulfides are converted to S-sulfo groups during SO_2 steeping; amounts of -SH, -SS-, and -SSO₃- in products were determined. Coextrusion of CGM with soybean meal gave highly textured nutritious products having poultry meat appearance. Optimum texture and color were achieved after defatting and decolorizing CGM with ethyl acetate. A juice extractor separated sugar beets into 68% sugar-containing juice, potentially useful in fermentation and for sugar production, and 32% pulp.

Publications:

WU, Y. V., SEXSON, K. R., and WALL, J. S. 1983. Reverse osmosis of soluble fraction of corn stillage. *Cereal Chem.* 60:248-251.

WALL, J. S., BOTHAST, R. J., LOGODA, A. A., SEXSON, K. R., and WU, Y. V. 1983. Effect of recycling distillers' solubles on alcohol and feed production from corn fermentation. *J. Agric. Food Chem.* 31:770-775.

BOOKWALTER, G. N., KWOLEK, W. F., WARNER, K. A., WALL, J. S., WU, Y. V., and GUMBMANN, M. R. 1983. Investigations on the use of distillers' grains...Food for Peace...Final Report to U.S. Congress, NRRC, Peoria, Illinois, 38 pp.

NEUMANN, P. E., WALKER, C. E., and WANG, H. L. The effects of fermentation with proteolytic fungi Aspergillus oryzae and Rhizopus oligosporus on the nutritional quality of corn gluten meal protein. *J. Food Sci.*, in press.

3. Isolation, Purification, and Characterization of Corn, Alcohol-Soluble Proteins (Zeins) (Cooperative Agreement - Virginia Polytechnic Institute and State University)

Methods of sequential extraction of proteins from corn endosperm were explored to maximize yield and purity of different solubility fractions of zein and reduced glutelin protein. The zein and separated alcohol-soluble reduced glutelin (ASG) proteins were further fractionated by ion exchange chromatography on sulfoethyl cellulose, sulfopropyl-Sephadex and phosphocellulose columns. These chromatography procedures resulted in partial fractionation of zein with separation of 22,000 dalton components from the 20,000 dalton proteins. Proline-rich water-soluble (WS) ASG's and methionine-rich water-insoluble (WI) ASG's were also resolved to yield some fairly pure proteins as revealed by isoelectric focusing (IEF). Further purification was achieved by preparative IEF. Peptide mapping of the purified proteins established that α -zein, WS-ASG and WI-ASG had very different structures. These findings were confirmed by N-terminal amino acid analyses of these proteins conducted at NRRC which established that WS-ASG had a unique hexapeptide sequence that was repeated several times. The purified proteins were injected into rabbits to induce antibodies. An enzyme-linked immunoassay was developed which established that antibody to a purified zein reacted with all other alcohol-soluble corn proteins, while the WS-ASG antisera was specific for that protein. The m-RNA's coding for WS-ASG were isolated and employed to synthesize this protein in an in vitro system. Efforts to clone the c-DNA for the ASG protein are in progress.

Publications:

ESEN, A., BIETZ, J. A., PAULIS, J. W., and WALL, J. S. 1981. Fractionation of alcohol-soluble reduced corn glutelins on phosphocellulose and partial characterization of two proline-rich fractions. *Cereal Chem.* 58:534-537.

ESEN, A., BIETZ, J. A., PAULIS, J. W., and WALL, J. S. 1982. Tandem repeats in N-terminal sequence of a proline-rich protein from corn endosperm. *Nature* 296:678-679.

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CONROY, J. M. and ESEN, A. An ELISA assay for zein and other proteins using unconventional solvents for antigen absorption. *Anal. Biochem.*, in press.

4. Exorphin Activity of Separated Peptides of Wheat (Cooperative Agreement - New York Hospital-Cornell Medical Center)

Approximately 110 peptide samples derived by enzymic hydrolysis of purified wheat gliadin proteins were analyzed for exorphin activity by a competitive binding test in fresh rat brain radiolabelled with tritium-containing dihydromorphine (DHM). Morphine was used to construct a standard DHM displacement curve, against which opioid-like activity of wheat peptides was compared. Aliquots of radiolabelled tissue homogenates were added to tubes containing morphine or peptide fractions, or to blank tubes; determinations were performed in triplicate at 25°C for 1 hr. Samples were filtered, and analyzed for radioactivity. While many samples had little or no activity; several fractions were very active; as little as 0.2-1.0 mg of gliadin peptide was equal to one nanomole of DHM. Since most peptide fractions are still heterogeneous, higher activities may be expected upon further purification.

FERMENTATION LABORATORY

C. W. Hesseltine, Chief

A. Agricultural Microbiology Research

R. W. Detroy, Research Leader

1. Molecular Genetics Technology for Microbial Production of Plant Polysaccharide-Degrading Enzymes (R. W. Detroy)

In developing cloning strategies for the transformation of the primary genes involved in pentose utilization, a number of selected mutants have been isolated being deficient in xylose utilization. The PM 213 mutant is xylose negative/glucose positive and deficient in xylitol dehydrogenase activity but xylose reductase positive. Cell extracts of xylose induced PM 213 overproduces the reductase and xylitol as end product. The first two enzymatic steps in the transformation of xylose by Pachysolen tannophilus have been defined. The NADPH-linked D-xylose reductase and NAD-linked xylitol dehydrogenase enzymes have been isolated and purified by affinity and ion exchange chromatography. The reductase is capable of converting xylose, arabinose, and galactose to their respective sugar alcohols. Specific antibody has been produced to the reductase to use as a selective probe against recombinant clones. The enzyme responsible for the conversion of cellodextrins (cellulosic intermediates) to glucose have been purified from Candida wickerhamii. The native molecular weight is 200,000 and a glycoprotein and capable of complete hydrolysis of cellodextrins G6 and G2 (in vitro). The C. wickerhamii organism was tested against cellodextrins derived by acid hydrolysis and fermented the carbohydrate at 80-percent yields to ethanol directly.

Publications:

BOLEN, P.L. and SLININGER, P.J. Continuous culture selection of mutant strains of Pachysolen tannophilus capable of rapid aerobic growth on D-xylose. In Developments in Industrial Microbiology. In press.

FREER, S.N. 1983. Fungal nucleic acids. In Secondary Metabolism and Differentiation in Fungi. Eds., J.W. Bennett and A. Ciegler. Vol. 5, Marcel Dekker, Inc., New York and Basel.

GOULD, J. MICHAEL and FREER, S.N. High-efficiency ethanol production from lignocellulosic residues pretreated with alkaline H₂O₂. Biotechnol. Bioeng. In press.

KURTZMAN, C.P. and BOLEN, P.L. 1983. Fermentation of xylose from biomass. Biotechnology 1:493.

WING, R.E. and FREER, S.N. Use of trifluoroacetic acid to prepare cellodextrins. Carbohydr. Polymers. Accepted.

2. Microbiological and Enzymatic Procedures for Conversion of Agricultural Residues and Biomass (R. W. Detroy)

Hydropulping, dilute acid pulping, alkali extraction, alkali extraction after hydropulping, and alkaline hydrogen peroxide were evaluated for their effectiveness in removing pentosans and lignin from wheat straw and for increasing accessibility of the cellulose to enzymatic hydrolysis. Pentosans were removed preferentially and in greater yield by alkali. Dilute acid pulping provided 5-carbon sugars (mostly xylose) directly. Although all treatments increased cellulose accessibility, alkaline hydrogen peroxide removed lignin more completely and was the most effective. CMCase was produced by 7 strains of Spicellum roseum in both liquid and wheat bran solid substrate cultures. High yields (90%) of ethanol were produced continuously from both saccharified corn starch and commercial glucose for 10 days of an 18 day fermentation at 30°C with calcium alginate entrapped Saccharomyces cerevisiae. Candida wickerhamii encapsulated into calcium alginate beads (1×10^9 cells/bead) converted cellobiose to glucose continuously for 14 days; however, ethanol yield was only 50-80% basis glucose. C. wickerhamii and Saccharomyces cerevisiae encapsulated together increased ethanol production by 10%.

Publications:

DETROY, R. W., CUNNINGHAM, R. L., and HERMAN, A. I. 1982. Fermentation of wheat straw hemicellulose to ethanol by Pachysolen tannophilus. Biotechnol. Bioeng. Symp. No. 12:81-89.

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DETROY, R. W. and ST. JULIAN, G. 1983. Biomass conversion: Fermentation chemicals and fuels. CRC Crit. Rev. Microbiol., 10(3):203-228.

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VAN CAUWENBERGE, J. E., LAGODA, A. A., BOTHAST, R. J., ALLEN, S. E., and FAHEY, G. C., JR. Captan-treated seed corn in alcohol production. J. Agric. Food Chem., in press.

SILMAN, R. W. Ethanol production by Zymomonas mobilis in fed-batch fermentations. Biotechnol. Bioeng., in press.

3. Feeding Trials of Animal Waste-Grain Fermented Feeds Using Recycled Waste (Cooperative Agreement - University of Illinois)

Young cattle (14/group) were fed for an 82-day period. Test diet was corn-waste ferment (waste equals 14% dry matter) amended with urea after fermentation to match nitrogen level in corn-soy diet of controls. During feeding trials, two test animals showed displaced abomasa. Diet was then changed to include 20-percent silage as compared with 15-percent silage used in standard diet. At the end of feeding trial, dry matter intake was 19.8 pounds/day (control) vs. 16.6 pounds/day (test animals), daily gain 2.4 pounds/day (control) vs. 2.15 pounds/day (test animals) and feed/gain of 8.3 (control) vs. 7.7 (test animals). Using fermented product, approximately 20-percent less grain is required to produce weight gains equivalent to animals on control diet. In a 22-day test period, pigs accepted a diet consisting of 40-percent cattle-waste corn ferment and 60-percent standard diet. Average dry matter intake, daily gain, and feed/gain were slightly lower than the control animals. In part, this may be caused by unsatisfactory flow of the moist test diet in self feeders.

4. Bioconversion of Agricultural Processing Wastes by Cellulolytic Fungi (P. L. 480 Grant - Boris Kidric Institute of Chemistry, Ljubljana, Yugoslavia)

The bioconversion of waste material remaining after apple brandy distillation was investigated. Different cellulolytic fungi were tested for their ability to convert organic substances into microbial biomass. In previous experiments mostly a cellulolytic Aspergillus niger strain was used. In the present experiments besides A. niger two Trichoderma strains (T. reesei and T. viride) and one basidiomycete (Pleurotus ostreatus) were used. The fungal strains were cultivated in substrates prepared on the apple slops with the addition of a mineral salt solution or another nitrogen source. The microbial growth, the filtrability of the broth and the chemical oxygen demand in the filtrate as well as the cellulolytic activity were followed during fermentation. By means of the fermentation process it was possible to reduce the filtration time and the chemical oxygen demand as well as to improve the raw protein contents in the filter cake and the cellulolytic activity of the filtrate. By mutation with gamma irradiation we succeeded in improving the cellulolytic activity of the parent A. niger strain.

5. Degradation of Lignin and Lignocellulosic Material by Mixed Cultures of Microorganisms (P. L. 480 Grant - University of Zagreb, Yugoslavia)

The first year on this project was devoted to organization of the research team, definition of negotiated objectives in conjunction with the Cooperating Scientist, definition of substrate composition and selection of appropriate microbes and microbiological methods. The autohydrolysis/chemical pretreatment of corn stover has been started but will require further optimization prior to the subsequent use of the Trichosporon system for lignin degradation. Characterization of the resulting lignins by IR spectroscopy after biological modification by Trichosporon cultures indicates structural changes coupled to the isolation of monomeric ring compounds. This initial evidence on biological delignification of corn stover lends well to the generation of modified cellulosic/hemicellulosic components for producing subsequent sugars and fermentation end products.

6. Fermentative Utilization of Cane Sugar Bagasses (P. L. 480 Grant - National Research Centre, Cairo, Egypt)

This research resulted in the isolation of two interesting microorganisms which are being tested at NRRC for the bioconversion of wheat straw to fuel and feedstuffs. The data generated in their country should now be most useful to them commercially for upgrading sugarcane bagasses as feedstuffs.

B. ARS Culture Collection Research

C. P. Kurtzman, Research Leader

1. Rapid Characterization of Yeasts Through Genetic and DNA/DNA Hybridization and Computer Analysis (C. P. Kurtzman)

Hansenula bimundalis var. bimundalis and H. bimundalis var. americana are heterothallic taxa showing 20 percent intervarietal DNA base sequence relatedness. Limited intervarietal conjugation occurred, but there were no fertile progeny. This is consistent with our earlier observations with other yeasts and indicates the two varieties actually to be closely related species. By contrast, H. anomala var. anomala and H. anomala var. schneegii showed nearly 100 percent relatedness and represent the same taxon. Comparisons between the wheat smut fungi Tilletia caries, T. controversa, and T. foetida showed 98 to 100 percent DNA relatedness indicating the three taxa to represent pathogenic races of a single species. DNA relatedness between mycotoxigenic and koji molds gave: Aspergillus flavus + A. oryzae, 97 percent; A. parasiticus + A. sojae, 85 percent; and A. flavus + A. parasiticus, 65 percent. These data indicate all four taxa to be varieties of the same species.

Publications:

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2. Germ Plasm Bank of Microorganisms for Research on Plant Residue Utilization (C. P. Kurtzman)

The Agricultural Research Service Culture Collection continued operation as an International Depository Authority and initiated a plan to charge a \$500 user fee for all deposits made to fulfill patent requirements and a \$20 fee for all new patent strains released for distribution. DNA was isolated on all of the strains listed in the Pridham 1976 Key as well as selected strains of Streptomyces albus and S. griseus. Continued DNA/DNA reassociation studies on these strains show that this method produces reliable data and that these strains are not related. Strains representing 20 described Micromonospora species have been acquired from other culture collections for integration into the ARS culture collections. Morphological and physiological data constituting a series of 48 unit characters has been collected for a set of 41 Micromonospora strains. The utility of this primary battery of test for distinguishing between Micromonospora taxa has been evaluated. DNA reassociation and extensive characterization studies revealed that strains of Bacillus circulans could be separated into a group representing B. circulans sensu stricto and 9 other genetically and phenotypically distinct groups. The 9 groups included hitherto undescribed species, or previously, but inadequately, described species. Inclusion of unrelated species into the taxon accounted for the heterogeneity of the species B. circulans. DNA reassociation and extensive characterization studies demonstrated that "Bacillus pulvifaciens" was a genetically and phenotypically distinct taxon. Hence, the revival of the Bacillus pulvifaciens was proposed to restore its standing in bacteriological nomenclature.

Publications:

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ANGEL, L. and WICKLOW, D.T. 1983. Coprophilous fungal communities in semi-arid to mesic grasslands. Can. J. Bot. 61:594-602.

WICKLOW, D.T. and ZAK, J.C. 1983. Viable grass seeds in herbivore dung from a semi-arid grassland. Grass Forage Sci. 38:25-26.

3. Soybean Foods of Oriental Type (H. L. Wang)

Glucamylase of Amylomyces rouxii, which is found only in the starters used for starch fermentation in the Orient, was isolated and characterized. The homogeneity of the enzyme was judged by electrophoresis and reverse-phase high-pressure liquid chromatography. The enzyme is a glycoprotein, has an optimum pH 4.5, optimum temperature of 60°C, a molecular weight of 55,600, and Km value of 15.8, 27.6, and 16.8 mg/ml for soluble starch, glycogen, and amylopectin, respectively. The enzyme hydrolyzes soluble starch and amylopectin at about the same rate converting these substrates almost completely to glucose, whereas the initial rate of glucose formation from glycogen is slower with 82-percent conversion observed. Unlike the other fungal glucamylases which were found from submerged cultures to exist in multiple forms, A. rouxii glucamylase, isolated from solid substrate fermentation, displayed only one form.

Publications:

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WANG, H.L. Technical aspects of whole soybean use. Proc. Iowa State Univ. Nutritional Sci. Council Symp. "Roles of Cereals and Legumes in the Food Supply." Academic Press. Accepted.

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4. Origin and Ecology of Mycotoxin-Producing Fungi in Grain (D. T. Wicklow)

Domesticated strains of yellow-green aspergilli differ from "wild" strains primarily because long-term domestication results in the loss of certain taxonomic characters having adaptive value in nature. Aspergillus oryzae and A. sojae, known only from Oriental fermented foods (e.g., koji molds), are classified as varieties of the wild species A. flavus and A. parasiticus. A. niger inhibits aflatoxin production by A. flavus on autoclaved corn kernels by a mechanism of lowering substrate pH (to approximately pH 3.1). Conidium germination in nontoxic domesticated strains of yellow-green aspergilli occurred 3 hours sooner, on the average, than for wild strains that produce aflatoxins.

Publications:

WICKLOW, D.T. and SHOTWELL, O.L. 1983. Intrafungal distribution of aflatoxins among conidia and sclerotia of Aspergillus flavus and Aspergillus parasiticus. Can. J. Microbiol. 29:1-5.

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WICKLOW, D.T. 1983. Adaptation in wild and domesticated yellow-green aspergilli. In Toxigenic fungi--their toxins and health hazard. Y. Ueno (Ed.). Elsevier, The Netherlands. (in press)

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HESSELTINE, C.W. 1983. Foreward. In Trichothecenes: Chemical, biological and toxicological aspects. Y. Ueno, ed., Kodansha Ltd., Tokyo, and Elsevier, Amsterdam and New York. pp. xi-xii.

5. Microbial Species Interactions and Development of Aflatoxin in Preharvest Corn (Cooperative Agreement - University of Wisconsin)

Aspergillus flavus formed large numbers of sclerotia in wound-inoculated preharvest corn produced in a controlled environment room (Biotron, University of Wisconsin). The majority of the sclerotia were recovered from wounded kernels; intact kernels located adjacent to the wounded kernels were all infected with A. flavus but supported few sclerotia, and these formed exclusively on the pericarp surface.

Publication:

WICKLOW, D.T. and HORN, B.W. 1984. Aspergillus flavus sclerotia form in wound-inoculated corn. Mycologia. Accepted.

6. Mycotoxins in Food and Feedstuffs (P. L. 480 Grant - Pakistan Council of Scientific and Industrial Research, Lahore)

The survey of aflatoxin in foods and feeds, using standard aflatoxin assays, can be summarized as follows: aflatoxin was absent in 200 wheat samples, absent in 54 rice samples, but 14 percent of the maize samples, 5 percent of the peanuts, and 21 percent of the walnut samples contained aflatoxin. Among fruits, no aflatoxin was found in figs (eight samples), raisins (18), coconut (21), almonds (14), and dry dates (2). In 234 samples of pulses and beans, no aflatoxin was present. In 81 feed samples, 17 percent were positive. In oilseed meal and cakes (128 samples), 5 percent showed aflatoxin from 24 to 160 ppb. A microbiological

examination of all the above samples for Aspergillus flavus was made on the same samples. This mold was absent from wheat, oats, and rice, but was detected in some samples of maize, groundnuts, walnuts, Pinus gardenia, poultry feed, oilseed cake, and sunflower cake. In general, the same aflatoxin contamination picture emerges as reported in the U.S. Corn and poultry feeds show the most contamination. The highest level of aflatoxin B1 was 800 ppb in corn. The two next highest levels were found in walnuts (400 ppb) and dried bread (640).

C. Microbial Biochemistry Research

M. E. Slodki, Research Leader

1. Polysaccharides in Specific Associations of Nitrogen-Fixing Microbes with Plants (M. E. Slodki)

Soil bacilli that excrete depolymerases active on 4-O-methyl-D-glucurono-L-rhamnan (MGR) and the galactose/galacturonic heteropolysaccharide (G/GH) of Rhizobium japonicum substrains were isolated from enrichment culture. Enzyme properties were studied and the reaction products were characterized. Inhibitors of binding of the uronic acid-specific lectin to MGR were D-glucuronic acid and its 4-O-methyl ether as well as their alpha, beta-methyl glycosides and the tetrasaccharide repeat unit obtained by enzymolysis of MGR. Presence of this new lectin in all varieties of soybean tested lends credence to a lectin-polysaccharide recognition mechanism. Considerable purification of the lectin was obtained by affinity chromatography in which either the tetrasaccharide or a partial hydrolyzate of MGR were used as ligands. Structural microheterogeneities in G/GH were confirmed and were reflected in differential susceptibility to depolymerase action. A number of tropical rhizobia were found that form either MGR or G/GH and also nodulate soybeans. Variants of MGR were found in which the L-rhamnose residue bearing the 4-O-Me glucuronic branch is 1,2,4- rather than 1,3,4-tri-O-substituted. These findings may explain the versatility of tropical rhizobia. Experiments were completed on reaction of phenacyl bromide with polysaccharide uronic acid to form reducible phenacyl esters. The method offers speed, ease of reaction and detection of sterically hindered uronic acid residues.

Publications:

CADMUS, M.C. Salt-tolerant microbial xanthanase and method of producing same. U.S. Patent 4,410,625. October 18, 1983.

CADMUS, M.C. and KNUTSON, C.A., JR. Production of high-pyruvate xanthan gum on synthetic medium. U.S. Patent 4,394,447. July 19, 1983.

DOMBRINK-KURTZMAN, M.A., DICK, W.E., JR., BURTON, K.A., CADMUS, M.C., and SLODKI, M.E. 1983. A soybean lectin having 4-O-methyl-D-glucuronic acid specificity. Biochem. Biophys. Res. Commun. 111:798-803.

SLODKI, M.E. and CADMUS, M.C. 1983. Production, composition and biodegradation of xanthan gum (Polysaccharide B-1459). U.S. Dept. Energy CONF-8205140, pp. 3-5.

2. Physiology of Nitrogen-Fixing Blue-Green Algal and Rhizobial Symbioses with Plants (J. W. Newton)

A sensitive method was developed to quantitate cyanophycin granule polypeptide (CGP) in blue-green algae and *Azolla*. The nitrogen-rich polymer was separated from proteins in extracts by high-pressure liquid chromatography using a large porosity reverse phase system. In senescent algae, where it is formed, CGP is very stable and is not broken down in extracts even when algal proteins autolyse. From extracts of *Azolla*, however, CGP is obtained in relatively low yield and chromatographically appears to be altered. ¹⁴C CGP was prepared for use as a substrate for degradation studies. Contrary to published reports, there was no solubilization of radiolabel by soil bacilli or by blue-green algae incubated under various conditions. An assay for soybean root nodulations using plastic plant-growth pouches was established to further characterize subspecies of *Rhizobium japonicum*. Thus far, all strains that produce the 4-O-methyl glucuronorhamnan but one also form effective nodules. In the growth pouches, Tan + mutants of *R. japonicum* NRRL L-259, which nodulate effectively, cause degeneration of the soybean tap root where it exits the paper trough. When kinetin is added, side roots proliferate and the plant survives. Additional mutants of L-259 were isolated. These differ from Tan + mutants in ability to grow on either tryptophan or alpha-ketoglutaric acid as C sources and NH₄ + as N source.

Publications:

KANESHIRO, T., BAKER, F.L., and JOHNSON, D.E. 1983. Pleomorphism and acetylene-reducing activity of free-living Rhizobia. J. Bacteriol. 153:1045-1050.

KANESHIRO, T., SLODKI, M.E. and PLATTNER, R.D. 1983. Tryptophan catabolism to indolepyruvic and indoleacetic acids by *Rhizobium japonicum* L-259 mutants. Current Microbiol. 8:301-306.

D. Mycotoxin Analytical and Chemical Research

O. L. Shotwell, Research Leader

1. Aflatoxin and Other Mycotoxins in Corn and Other Cereal Grains (O. L. Shotwell)

A study was made of deoxynivalenol (DON) in 1982 soft red winter wheat from Missouri. Of 44 samples analyzed, 64% had less than 0.3 ppm DON; 34% had 0.3-1.0 ppm; and 1 sample had 1.04 ppm. Correlations of grading factors, *Fusarium* damage, and germination with DON levels have been

made. Samples of flour prepared from 1982 hard red winter wheat grown in areas of Kansas and Nebraska that had scabby wheat were analyzed for DON. Of 120 samples, 26% had more than 1.0 ppm. The mean level of DON in flour samples was 0.76 ppm and 1.50 ppm in 1982 wheat grown in the same area. A cooperative feeding trial on turkeys was conducted with J. L. Richard, National Animal Disease Center, Ames, Iowa. Turkeys were fed rations containing 0, 50, and 150 ppb aflatoxin for 13 weeks. Some turkeys were sacrificed, some were placed on aflatoxin-free rations and some continued on contaminated diets for 2 weeks before sacrifice. Turkeys fed 15 weeks on rations containing 50 and 150 ppb aflatoxin had 0.6 and 0.18 ppb aflatoxin B₁ and 0.05 and 0.15 ppb aflatoxin M₁, respectively in the livers. Isolates of Fusarium graminearum from field-inoculated corn known to produce DON were tested for toxin production after 1 year's storage under liquid nitrogen. Capability of the strains to produce DON was not affected. Dust samples of varying particle sizes were collected as corn was delivered at an elevator. Of the dust particles collected, 22% were respirable. Analytical methods have been developed to determine aflatoxin B₁ in 1.0-, 0.0-, and 0.01-g samples of corn and dust from corn.

Publications:

BENNETT, G.A., STUBBLEFIELD, R.D., SHANNON, G.M., and SHOTWELL, O.L. 1983. Gas chromatographic determination of deoxynivalenol in wheat. J. Assoc. Off. Anal. Chem. 66:1478-1480.

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2. Development of Improved and Simplified Methods for Trichothecene Mycotoxins
(Grant - Iowa State University)

Mass spectral fragmentation patterns of trichothecenes and various esterified derivatives were obtained by electron impact and chemical ionization modes. The preparation of more volatile derivatives for GLC analysis was studied. A method of cleanup of trichothecenes in different matrices by volatilization in an induction furnace at 520°C for 15 seconds was developed that permitted more than 80-percent recovery of diacetoxyscirpenol and T-2 toxin. The volatilized toxins were trapped in aluminum tubes and the contents were analyzed by GLC. TLC analyses on alumina were studied. Because vomitoxin fluoresces in this system, submicrogram sensitivity is possible with combined fluorometry/alumina TLC. Sample cleanup methods utilizing Sep Pak C18, florisil, silica, and XAD resins all improve the quality of extracts prior to analysis. Ultraviolet spectra of trichothecenes were recorded and differences were utilized in developing an HPLC analysis with UV detection.

3. Application of the Trickle Ammonia Process to Drying Corn at Ambient Temperatures (Cooperative Agreement - University of Minnesota)

High-moisture corn (25.4, 26.5%) from two crop years (1981, 1982) has been dried to 13.4, 14.5% moisture by the trickle ammonia process (TAP). Movement of the ammonia through the bin was followed by cartridges containing adsorbant that changed from white to purple when exposed to ammonia. Detailed studies on the microflora content indicated that ammonia was effective in controlling fungi. Studies on feeding corn dried by TAP to dairy cattle indicated there was no adverse effect on feed intake or milk production. The process has been described and/or demonstrated to farmers and elevator owners at 47 conferences, meetings and workshops. Scientific papers and a bulletin containing information on the use of TAP on farms and at elevators are being prepared.

E. Mycotoxin Microbiology and Biochemistry Research

M. D. Grove, Research Leader

1. Metabolites of Toxin-Producing Fungi Found in Corn and Other Cereal Grains (M. D. Grove)

The 6-methoxydihydrofurobenzofuran portion of aflatoxin was synthesized in trace amount by thermolysis of the ethyl carbonate ester. Six derivatives of T-2 toxin were prepared and tested for feed refusal activity using the mouse drinking-water bioassay. Either acetylation or hydrolysis of T-2 toxin gave compounds with slightly diminished activity. Catalytic hydrogenation of T-2 tetraol decreased activity by half. Reduction of the spiro epoxide group by lithium aluminum hydride resulted in elimination of the refusal response. A decontamination procedure consisting of mild alkaline hydrolysis of T-2 toxin to T-2 tetraol followed by a thermal hydration gave a mixture of epimeric hydrates and a tetrahydroxyketone in 70- and 17-percent yield, respectively, which are devoid of refusal activity. The structure of the *Fusarium roseum* and *F. tricinctum* metabolite that causes conidia of *Penicillium* and *Aspergillus* species to swell has been established as a mixture of three cyclodepsipeptides. The amino acid sequence is threonine-alanine-alanine-glutamine-tyrosine plus either leucine, isoleucine, or valine. The N and C termini are linked into the cyclic structure via peptide and lactone bonds with 3-hydroxy-4-methyltetradecanoic acid. A new metabolite isolated from *F. moniliforme* by HPLC was identified as 10,11-dihydroxyfusaric acid. Progress on characterization of an antimicrobial phytotoxic product of *F. tricinctum* suggests the compound is a unique triterpenoid derivative. Production studies of vomitoxin were continued and quantities were supplied to cooperators for toxicological studies. Toxin-producing strains of *F. moniliforme* were the only molds isolated from chicken feed reported to cause feed refusal. This feed did not contain moniliformin or trichothecenes.

Publications:

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COLE, R.J., DORNER, J.W., LOMAX, L.G., VESONDER, R.F., BURMEISTER, H.R., and STUART, B.P. Comparative effects of long-term feeding of T-2 toxin and deoxynivalenol to broiler chickens. Toxicol. Appl. Pharmacol. In press.

COTE, L.M., REYNOLDS, J.D., VESONDER, R.F., BUCK, W.B., SWANSON, S.P., COFFEY, R.T., and BROWN, D.C. Incidence of vomitoxin in midwestern feed grains and associated health problems in livestock. J. Am. Vet. Res. In press.

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PETERSON, R.E. and GROVE, M.D. 1983. Isolation of xanthomegnin from Penicillium viridicatum by preparative high-pressure liquid chromatography. Appl. Environ. Microbiol. 45:1937-1938.

STUART, B.P., COLE, R.J., WALLER, E.R., and VESONDER, R.F. Proventricular hyperplasia in broiler chickens. J. Environ. Pathol. Toxicol. In press.

VESONDER, R.F. 1983. Natural occurrence in North America. In Trichothecenes: Chemical, Biological and Toxicological Aspects. Developments in Food Science, Vol. 4. Y. Ueno, ed. Elsevier Press, Amsterdam, Netherlands. pp. 210-217.

2. Evaluation of Safety of Soybean Foods--Tempeh, Tofu, and Miso (Cooperative Agreement - University of Wisconsin)

The bacterial food-poisoning species used in this project are Clostridium botulinum, Yersinia enterocolitica, Salmonella typhimurium, and Staphylococcus aureus. S. typhimurium and Y. enterocolitica were killed in 24 hours in low-salt miso when inoculated with 10^2 /g of cells. The factors affecting death are not known. When low-salt miso was inoculated with spores of C. botulinum and incubated at 15° and 25°C for 4 weeks, no toxin was produced. On the other hand, tofu was an excellent substrate for the development of S. typhimurium and Y. enterocolitica. Population densities reached were 3×10^8 for the former and 2×10^8 for the latter in 2 weeks at 25°C. Y. enterocolitica grew slowly on tofu in the refrigerator (5°C) and in 14 days the population had increased 10,000-fold. Tempeh was also found to be a good substrate for C. botulinum with toxins formed in inoculated tempeh cakes at 30° in 3 days. In all the studies, more than one known toxin-producing strain was used.

HORTICULTURAL AND SPECIAL CROPS LABORATORY

E. B. Bagley, Chief

A. Composition and Characterization Research

C. R. Smith, Jr., Research Leader

1. Chemicals in Tall Fescue Affecting Livestock Health and Forage Utilization (S. G. Yates)

A method to detect and measure ergot cyclo alkaloids in tall fescue is being evaluated. Ergotamine spiked into tall fescue samples at levels as low as 0.01 ppm could be detected directly from a single chloroform/ammonia extract of the defatted fescue sample by MS/MS. In a separate study, ergot sclerotia-tall fescue seed screenings were blended with tall fescue samples at 100, 50, 10, 1, and 0.1% levels. The ergot cyclo alkaloids were detectable in extracts of all samples except the controls. Recovery of the alkaloids was satisfactory down to the 1% blend. In the 0.1% blend the S/N for ergotamine was only an estimated 5 to 1. Because of matrix problems larger aliquots of extract could not be analyzed without a sample cleanup.

Publications:

BOLING, J.A., HEMKEN, R.W., BUSH, L.P., BUCKNER, R.C., JACKSON, J.A., JR., and YATES, S.G. 1983. Role of alkaloids & toxic compound(s) in utilization of tall fescue by ruminants. Proc. XIV Int. Grasslands Conf. Sect. IX, pp. 722-725, Westview Press.

PLATTNER, R.D., YATES, S.G., and PORTER, J.K. 1983. Quadrupole mass spectrometry/mass spectrometry of ergot cyclol alkaloids. J. Agric. Food Chem. 31(4):785-789.

2. Bioassay of Chemical Constituents of Tall Fescue Forage (Cooperative Agreement - University of Missouri)

Tall fescue anions that were held weakly on an anion exchange resin (Fraction A) were isolated from a toxic tall fescue sample and given IP to calves. This fraction produced a weight loss and a reduced weighted-average-coronary band-temperature (WACBT); other clinical signs of fescue foot were mild. This indicates that a factor that reduces the temperature of the calves' feet is present in this fraction. Fifty %-ethanol extracts of a toxic KY-31 forage and an endophyte-infected seed were given intraruminally (IR) to calves. These extracts produced a weight loss, a reduced WACBT, plus other clinical signs of fescue foot (lameness and swelling in the feet, inflammation of the coronary bands, and tissue necrosis). This shows that IR route of administration gives

clinical signs of fescue foot with a maximum dose equivalent to 10 pounds of hay or seed per day in a 12-day trial. The results also indicate that the total fescue syndrome is due to several factors. 2,4-Dihydroxy butyrolactone (DHBL) containing NaBr (from preparation and neutralization steps) produced loss of weight and a reduced WACBT. When dosed IP at a maximum of 16 g of each component per day during a 12-day test, NaBr by itself produced no effect. This indicates that DHBL, which is a component of tall fescue, may be one factor that causes a reduction of the temperature of calves' feet.

Publications:

YATES, S.G., GARNER, G.B., and CORNELL, C.N. Steps leading to the testing of 2,4-dihydroxybutyric acid as a factor in toxicity of tall fescue to cattle. Workshop Problems Associated Util. Fescue by Cattle, Fayetteville, AR, 5/19-20/83.

3. Biologically Active Plant Constituents for Pest Control and Medicine (C. R. Smith)

In addition to sesbanimide A, the structures of two other bioactive compounds from Sesbania drummondii seed, sesbanimide B and sesbanimide C, have been elucidated. Unlike sesbanimide A, sesbanimide B exists as an anomeric mixture in solution. A new bioactive compound, a pre-ortho ester, has been identified as a constituent of Diarthron vesiculosum seed. The volatile oat constituents most attractive to the sawtoothed grain beetle (Oryzaephilus surinamensis) include the dimethyl esters of succinic, glutaric, and adipic acids. Two classes of cyanolipids (I and IV) exhibit a distinctive anesthetizing or paralyzing effect on O. surinamensis from which, however, they tend to recover in the absence of the cyanolipid.

Publications:

FERRIGNI, N.R., McLAUGHLIN, J.L., POWELL, R.G., and SMITH, C.R. Use of potato disc & brine shrimp bioassays to detect activity & isolate piceatannol as the anti-leukemic principle from the seeds of Euphorbia lagascae. J.Nat.Prod. In press.

FERRIGNI, N.R., PUTMAN, J.E., ANDERSON, B., JACOBSEN, L.B., NICHOLS, D.E., MOORE, D.S., McLAUGHLIN, J.L., POWELL, R.G., and SMITH, C.R., JR. 1982. Modification and evaluation of the potato disc assay and antitumor screening of Euphorbiaceae seeds. J. Nat. Prod. 45(6):679-686.

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4. Natural Toxicants in Horticultural Crops and Cruciferous Feeds (M. E. Daxenbichler)

Experimental evidence indicates that epithio specifier protein from Crambe acts upon a thioglucoside glucohydrolase allosterically in the process of converting glucosinolates to 1-cyanoepithioalkanes. A rapid, mild diffusion method has been devised for concentration of epithio specifier protein and other labile proteins. One-third of the required 150 g of toxicant (1-cyano-2-hydroxy-3,4-epithiobutane) is ready for a 2-year rat feeding study, expected to be initiated in Feb. 1984 through the cooperation of Dr. M. R. Gumbmann, Western Regional Research Center, Albany, California. Dr. Gumbmann has preliminary results, not yet reported, on feeding rats 3-methylsulfinylpropyl isothiocyanate and the corresponding nitrile (isolated at NRRC from Lesquerella gordonii seed).

Publications:

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SCHROEDER, W.P., DAXENBICHLER, M.E., SPENCER, G.F., WEISLEDER, D., and TOOKEY, H.L. 1983. 4-Hydroxy-3-methoxybenzylglucosinolate, a new glucosinolate of Brassica elongata. J. Nat. Prod. 46(5):667-670.

VANETTEN, C.H. and TOOKEY, H.L. 1983. Glucosinolates. In "Naturally Occurring Food Toxicants," M. Rechcigl, ed., pp. 15-30, CRC Press, Boca Raton, FL.

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YATES, S.G. Carrots: A new look at an old crop. The Herbarist. In press.

5. Major Fatty Acids from Indian Seed Oils and Their Possible Industrial Use (P. L. 480 Grant - Aligarh Muslim University, Aligarh, India)

Among the classes of fatty acid derivatives synthesized during the year are: products of reaction of mercaptopropanediol with unsaturated acids; fatty 1,3-dioxolanes; products of addition of N,N-dibromobenzenesulfonamide to epoxy acids; reaction products with mercaptoacetic acid. A new naturally occurring fatty acid, 9-hydroxydodecanoic acid, was identified as a component of Blepharis sindica seed oil. Seed oils of the following species were found to be repellent to the stored product pest Tribolium castaneum: Lochnera pusilla, Crotalaria juncea, Carum capticum, Sphaeranthus indiana, Cubeba officinalis, Exacum bicolor.

Publications:

GUPTA, R., AHMAD, M.S., AHMAD, F., and OSMAN, S.M. Nitrosochlorination of beta- and gamma-hydroxyolefinic fatty acid esters. Indian J. Chem. In press.

GUPTA, R., AHMAD, M.S., AHMAD, F., and OSMAN, S.M. Addition of N,N-dibromobenzenesulfonamide to beta-epoxy and beta(gamma)-hydroxy, gamma(beta)-chloroolefinic fatty acid derivative. III. Indian J. Chem. In press.

HASAN, S.Q., AHMAD, I., AHMAD, F., and OSMAN, S.M. Studies on nitroso-chlorination of olefinic fatty acids, Pt. I. JAOCS. In press.

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HASHMI, M., KHAN, M., AHMAD, M.S., AHMAD, F., and OSMAN, S.M. Oxidative cyclization of unsaturated fatty acids with lead tetraacetate (LTA). JAOCS. Accepted.

6. Inheritance of Glucosinolates in Crucifer Vegetables (Cooperative Agreement - University of Wisconsin)

Progress continued in development of rapidcycling stocks of selected Brassica sp. and Raphanus sativus; these are to be made available for breeding purposes. Recent attention has been devoted largely to Brassica oleracea and its crosses with crucifers of known glucosinolate content.

7. Biological Effects of Potential Toxicants from Glucosinolates (Cooperative Agreement - Colorado State University)

Mice are less sensitive to the effects of 1-cyanohydroxyepithiobutanes (CHEB) than rats. The early lesions in CHEB-intoxicated rats involve the lining cells of excretory channels, i.e., biliary epithelium and renal tubular epithelium. Further changes seem to be the result of regenerative activity that arises from the initial epithelial damage. Serum biochemical alterations are indicative of damage to bile ductule and renal tubular epithelial damage. There is the suggestion, from the 90 day mouse study, that nervous system damage may be associated with CHEB intoxication.

B. Instrumental Analysis Research

R. Kleiman, Research Leader

1. Isolation and Identification of Allelochemicals from Uncultivated Plants (R. Kleiman)

The biological screening of extracts from seed of 225 wild species revealed germination inhibitors in 21 of them. Selections from this group will be made for isolation and characterization of active compounds. Benzyl isothiocyanate, an active germination inhibitor of velvetleaf seed at the 4×10^{-4} M level did not affect corn even at moderately high concentrations such as 10^{-3} M but did affect soybean at the 10^{-3} M level. Soybeans were not affected at the 4×10^{-4} M level. The acetone extract of defatted Iva axillaris seeds was found to contain germination inhibitors tomentosin and ilicic acid. Other compounds, such as axivalin, had growth inhibitory properties. This work also resulted in the isolation of a new sesquiterpene, tentatively identified as the isovalerate ester

of ivaxillarin. Computer programs were written and data entered in order to have searchable files for future reference of germination inhibition data. In cooperation with plant breeders working in the new crop area, analyses for oil, protein, and fatty acids of seed were accomplished. Species included in this program were rape, crambe, Sapium, Sebiferum, Cuphea, and Vernonia.

Publications:

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WOLF, R.B., SPENCER, G.F., and KWOLEK, W.F. Inhibition of velvetleaf (Abutilon theophrasti) germination and growth by benzyl isothiocyanate, a natural toxicant. Weed Sci. In press.

2. Analysis for Improved Soybean Quality (R. Kleiman)

Over 19,000 soybean samples were submitted by public breeders and analyzed for oil and protein content. A pulsed NMR procedure was developed which determined oil content in whole soybeans. In cooperation with James Wilcox (ARS, Purdue University), fatty acid composition of 6000 samples, some only bean slices, were determined. Several samples were in the range of 3.0-3.5% linolenic acid. Some 2000 samples recently regrown from the germplasm collection at Urbana, IL, were analyzed for fatty acid composition. Antibodies to spinach acyl carrier protein were prepared in rabbits. These antibodies were shown to cross-react with acyl carrier protein from soybean and several other plant species. A novel and general radioimmunoassay for ACP was developed based on competition at the antibody combining site for [³H]-palmitoyl-acyl carrier protein. Measurement of acyl carrier protein levels at various stages of soybean seed development has indicated that de novo synthesis of fatty acid synthetase proteins can at least partially account for activation of the lipid storage process in oilseeds. Acyl carrier proteins have been partially purified from several plant and microbial species and their cross-reactivity with antibodies to spinach ACP examined. These experiments have revealed a high degree of conservation in the ACP structure during evolution. Acyl-ACP synthetase from E. coli was shown

to have the ability to acylate plant acyl carrier proteins. The K(m) and V(max) values for spinach ACP were remarkably similar to those for E. coli ACP.

Publications:

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3. Multi-Location Soil Nitrogen Program (R. Kleiman)

The improved nitrogen generation system was used to analyze over 1000 samples for $^{14}\text{N}/^{15}\text{N}$ ratios. These samples came from soil scientists in Minnesota, Kentucky, Nebraska, and Colorado. In an effort to stop purchase of expensive replacement bellows for the MAT 250 mass spectrometer, an alternate bellows system was devised in cooperation with a commercial firm that will allow inexpensive bellows replacements.

4. Detection and Quantitation of Mycotoxins and Natural Toxicants by Mass Spectrometry (R. D. Plattner)

A rapid MS/MS method was developed to detect Fusarium mycotoxins, DON, and zearalenone, in crude extracts of grain. This method was used to confirm the presence of DON in selected samples of hard red winter wheat grown in 1982 in Nebraska and Kansas. The accuracy and precision of quantitation obtained by MS/MS for various compounds was studied with and without an internal standard. The relationships between quality of data and such factors as source condition and analysis techniques were studied so that optimum conditions could be predicted. In an FDA collaborative study on identification of aflatoxin B1 by negative ion chemical ionization in peanuts, cottonseed, and ginger, our results were typical giving the same number of correct answers using standard procedures as for the other laboratories. Analysis of a duplicate set of samples by MS/MS without further clean-up gave perfect results and reliably identified this aflatoxin about an order of magnitude lower in concentration than the standard method. Ammoniation of artificially infected aflatoxin contaminated corn at ambient temperature and pressure followed by TLC and HPLC fractionation and MS/MS analysis led to

identification of aflatoxin D1 as an ammoniation product. MS/MS was used to study evaluation of ergot alkaloids in spiked hay. Quantitative recoveries of ergot were excellent down to 0.1% spiking when aliquots equivalent to 3 micrograms or less were analyzed. In larger samples, the matrix effects became fatal.

Publications:

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C. Physical Properties Research

E. B. Bagley, Research Leader

1. Basic Studies of Physical Properties of Soy and Cereal Based Food Ingredients (E. B. Bagley)

Starch gels (with and without hydrocolloids), dispersions of swollen starch granules and gelatin gels have been studied in both simple shear and torsional deformation. Both approaches give modulus values in good agreement. Torsional studies, in addition, give information on normal stresses generated in a direction perpendicular to the direction of deformation, a structurally sensitive property that heretofore has been neglected in studies of food materials. Analysis of the data showed that the Mooney-Rivlin model (a large deformation theory commonly used in polymeric systems) is inadequate to describe material response for reasons not clearly understood. Comparison of simple shear and torsion with compressional studies (commonly used in food systems to relate to textural properties) confirmed that there are fundamental theoretical

problems which need to be addressed. Additional work was carried out on viscosity behavior of dispersions of swollen starch granules. This work is unique in allowing a test of the Arrhenius relation between viscosity and volume fraction to be made in the volume fraction range 0.5-1.0. The theoretical relationship was adequate to describe the behavior.

Publications:

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NAVICKIS, L.L. and BAGLEY, E.B. 1983. Yield stresses in concentrated dispersions of closely packed, deformable gel particles. *J. Rheol.* 27(6):519-536.

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D. Plant Biochemistry and Photosynthesis Research

J. A. Rothfus, Research Leader

1. Plant Cell and Tissue Culture for the Bioproduction of Valuable Chemicals (N. E. Delfel)

Baseline resolution of a mixture of 7 triterpenes and beta-sitosterol (a common contaminant) was achieved by HPLC on silica gel under isocratic conditions using UV detection at 210 nm. Application of this method to hydrolyzed extracts from Chenopodium quinoa seeds gave oleanolic acid and hederagenin levels of 0.25 and 0.05% db, respectively, and levels one-tenth as high from callus cultures. These two triterpenes are the only ones present in quinoa saponins by GLC-MS. Cooperative studies with Purdue University have completely characterized one of the saponins

as a tridesmoside of hederagenin. This is the first report of a trisubstituted saponin in nature. Quinoa embryoids have been induced using a regeneration medium with 10^{-6} M abscissic acid and gibberellic acid and 5×10^{-6} M benzyl adenine. In one experiment normal-appearing embryos developed and germinated after 13 months. In related studies on algal protoplast production and fusion, high yields of protoplasts were obtained with *Chlorella vulgaris*, but other species have proven to be refractory, presumably due to sporopollenin in the cell wall.

2. Control of Plant Morphogenesis and Energy Transfer System Structure and Action in Crop Plants (J. A. Rothfus)

Atrazine binding to thylakoids is reduced in continuously illuminated versus dark-adapted samples, but binding site number is not changed by illumination. Decreased atrazine binding correlates directly with increased semiquinone form of PSII acceptor B in thylakoids treated photochemically to induce different levels of reduction. With flash excitation, herbicide binding oscillates with a cycle of two, being low on odd flashes when the semiquinone level is highest. These results support the quinone-herbicide competition theory of herbicide action. PSII reaction center assays by atrazine binding in dark-adapted thylakoids correlate with direct spectrophotometric measures of Q, but both methods give PSII concentrations double those measured by oxygen flash yield, which suggests multiple quinone-acceptor and herbicide-binding sites in each oxygen-evolving complex. Comparisons (cooperative with M. Duysen, North Dakota State University) of chloroplasts from normal and pigment-deficient soybeans have identified conditions under which variation due to genotype is small compared to that caused by light quality. Far-red chloroplasts have less pigment, which is enriched in light-harvesting complex (LHC) pigments. PSII/PSI ratios are elevated by far-red due to reduced amounts of PSI, but remaining PSI centers operate very efficiently. PSII/PSI ratios do not correlate with degree of grana stacking and far-red chloroplasts have few stroma thylakoids. Pigment-deficient genotypes in test plots also have fewer LHC's, higher PSII/PSI ratios, and much less grana stacking.

Publications:

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3. Metal-Binding Proteins of Field and Forage Crops and Their Regulatory Functions (L. C. Wang)

The incorporation of phosphorus from (γ -³²P)-ATP into TCA-precipitable proteins of pea shoot chloroplasts is light and calcium dependent and calmodulin dose responsive. In the presence of calcium ions and a phosphatase inhibitor, bovine calmodulin and a calmodulin-like protein from soybean seed both increase phosphorus incorporation some five-fold over untreated broken chloroplasts. Trifluoroperazine and EGTA, known inhibitors of calmodulin action, block this incorporation. Gel electrophoresis of dissociable components reveals eight clusters of phosphorylated proteins. Labelling is heaviest in those of less than 15 KD or more than 100 KD molecular weights and in a third cluster composed of two proteins of 30-33 KD molecular weight. These latter proteins are most susceptible to calmodulin-promoted incorporation. Similarly, the light-dependence of calmodulin-promoted incorporation is most evident in the 30-33 KD proteins.

4. Composition and Properties of Seed Lipids for Foods and Feeds (J. A. Rothfus)

Methyl esters of 5-cis- and 11-cis-eicosenoic acids exhibit different melting points depending on how they are cooled. Analyses suggest that specific polymorph formation follows first-order kinetics. Accordingly it is possible to identify structure-specific initiation temperatures for transformations occurring below either materials melting point. Melting energies for the highest melting polymorphs of these positional isomers are essentially the same indicating that both undergo the same type of perturbation upon melting. Apparent activation energies for transformations between penultimate and highest-melting forms are significantly different however. Paths from melt to solid and back to melt may not be reversible and may be directed by critical responses to conditions along the way.

Heat capacities for the four polymorphic forms of tristearin (C18) identified earlier evidence little or no difference from corresponding alpha and beta forms of trimargarin (C17) over a 140K temperature range, but there are significant differences between corresponding beta-prime forms. Heat capacities for the two beta-prime forms of C17 exceed those of C18 but they converge at a lower temperature than do those for the beta-prime forms of C18.

Publications:

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OILSEED CROPS LABORATORY

T. L. Mounts, Chief

A. Biochemical and Biophysical Properties Research

E. A. Emken, Research Leader

1. Biochemical Behavior of Isomeric Fats in Hydrogenated Soybean Oil
(E. A. Emken)

Mixtures of deuterium labeled triglycerides and fatty acids were administered to young male adults and lactating mothers in multiple labeled fat studies. The mixtures used contained various combinations of deuterium labeled palmitic, stearic, oleic, linoleic, 10c-, 10t-, 8c-, 8t-octadecenoic and 12c, 15t-octadecadienoic acid. Blood samples collected serially after feeding the labeled fats were extracted and individual lipid components separated and derivatized for analysis by gas chromatography-mass spectrometry (GC-MS). GC-MS data collection and processing methods were extensively modified and validated in order to analyze the wide variety of labeled and non-labeled fatty acids present in blood components. Oxidation rates for radioisotope labeled elaidic and oleic acid by human heart homogenates were compared to rat heart data. Oxidation of elaidic acid was similar to oleic acid for human heart homogenates but about 30% slower for rat heart. Various reactions and synthetic schemes were explored and evaluated for use in preparation of deuterium labeled polyunsaturated fats. Selected approaches were used to synthesize deuterium labeled linoleic and gamma linolenic acid.

Publications:

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B. Exploratory Organic Reactions Research

E. H. Pryde, Research Leader

1. Basic Chemistry of Vegetable Oils for Fuels and Alternative Chemicals (E. H. Pryde)

The high viscosity of vegetable oils is a major deterrent to their fuel and some industrial uses and may be reduced to more appropriate levels by microemulsification, conversion to simple esters, pyrolysis, or skeletal rearrangement of the fatty acid chain. Physical property characterization of a series of microemulsions formed with C-4 to C-18 alcohols, vegetable oils, and aqueous alcohols has shown these fuels to be promising candidates for engine tests (See B.2). Conversion to simple esters is carried out by transesterification, a reaction that was studied in detail to provide the basic information needed for industry or small cooperatives. Pyrolysis of vegetable oils produces a series of homologous alkanes, alkenes, cyclic compounds, and carboxylic acids as indicated by preliminary characterization by gas chromatography-mass spectroscopy. Preliminary work has been started on the characterization of skeletal rearrangement products, as in the commercially available isostearic acid. Opportunities were provided by a number of invited lectures and review book chapters to emphasize the importance of renewable resources (such as vegetable oils) as alternative fuels and chemical feedstocks.

Publications:

FREEDMAN, B., PRYDE, E.H., and MOUNTS, T.L. Variables affecting the yields of fatty esters from transesterified vegetable oils. J. Am. Oil Chem. Soc. (Accepted August 26, 1983).

PRYDE, E.H. 1983. The soybean: Its importance and use distribution. In: "The CRC Handbook of Processing and Utilization in Agriculture," Vol. 2: Part 2 Plant Products, edited by I. A. Wolff. CRC Press, Inc., Boca Raton, FL, pp. 3-14.

PRYDE, E.H. 1983. Nonfood uses of vegetable oils. In: "The CRC Handbook of Processing and Utilization in Agriculture," Vol. 2: Part 2 Plant Products, edited by I. A. Wolf, CRC Press, Inc., Boca Raton, FL, pp. 109-142.

PRYDE, E.H. 1983. Utilization of commercial oilseed crops. Economic Botany 37:459-477.

PRYDE, E.H. and OTEY, F.H. Renewable raw materials for the polymer industry. Polymer Yearbook. In press.

SCHWAB, A.W., NIELSEN, H.C., BROOKS, D.D., and PRYDE, E.H. 1983. Triglyceride/aqueous ethanol/1-butanol microemulsions. J. Dispersion Sci. Technol. 4(1):1-17.

2. Physical/Chemical Modification of Vegetable Oils for Diesel Fuel (E. H. Pryde)

Aqueous ethanol/vegetable oil microemulsions developed at NRRC have been studied in engine tests at the University of Illinois for heat release data and the 200-hour Engine Manufacturer's Association (EMA) screening test and at the Allis-Chalmers' Harvey, Illinois, plant under the supervision of North Dakota State University scientists for the EMA screening test. The heat release data indicate substantially greater amounts of pre-mix combustion and lesser amounts of flame diffusion combustion for overall faster and smoother burning compared to No. 2 diesel oil. In the 200-hour EMA tests, microemulsions containing more than 50% vegetable oil maintained power output for the duration of the test, and in this aspect they gave results superior to those obtained with 25/75 blends of sunflower oil in diesel oil. However, carbon buildup and gummy deposits in the cylinders and buildup of polymerized vegetable oil in the lubricating oil indicated potential engine failure if the screening test had been continued beyond the prescribed time. Similar problems arose with a methanol/vegetable oil microemulsion that was developed at NRRC and tested at the University of Illinois. These problems can be ascribed to incomplete combustion in the engine, and further work is needed to determine the effects of additives to improve combustion efficiency as well as to improve removal of carbon deposits. Simple esters of vegetable oils continue to show most promise as alternative fuels, and all have passed the EMA screening tests.

Publications:

PRYDE, E. H. 1983. Vegetable oil as diesel fuels: Overview. J. Am. Oil Chem. Soc. 60:1557-1558.

PRYDE, E. H. Chemicals and fuels from commercial oilseed crops. In "Fuels and Chemicals from Oilseeds: Technology and Policy Options," eds., E. B. Schultz, Jr., and R. P. Morgan. AAAS/Westview Press, in press.

PRYDE, E. H., and SCHWAB, A. W. 1983. Cooperative work on engine evaluation of hybrid fuels. Proc. Vegetable Oil as Diesel Fuel, Seminar III, Northern Agricultural Energy Center, Peoria, Illinois. ARM-NC-28, pp. 90-95.

SCHWAB, A. W., and PRYDE, E. H. 1983. Vegetable oil methanol systems as fuels for diesel engines. Proc. Vegetable Oil as Diesel Fuel, Seminar III, Northern Agricultural Energy Center, Peoria, Illinois, 1983. ARM-NC-28, pp. 85-89.

BAGBY, M. O. 1983. Seminar reflections and future research needs. Proc. Vegetable Oil as Diesel Fuel, Seminar III, Northern Agricultural Energy Center, Peoria, Illinois. ARM-NC-28, pp. 171-176.

3. Production Problems of the Oilseed Crop, Crambe (Crambe Abyssinica)
(Cooperative Agreement - Murray State University)

Three 10-acre fields of crambe were planted in Kentucky. Two fields planted early and subjected to a cold, wet spring, were harvested and placed in storage. The third field, planted in June, was lost to very hot, dry summer weather (seed pods were mostly empty due to poor seed set). Seed from these fields and replicated plots at Murray are being evaluated and seed composition determined. Weed control in all three production fields was satisfactory using no herbicide in one and either Basalin or Ramrod in the other fields. Fungicide trials were superimposed on the seed production fields using multiple applications of Maneb, Mancozeb, or Daconil. A consistent yield response from fungicide treatment was not observed in these tests, because hot, dry weather resulted in lower than normal incidence of Alternaria infection. In a pilot study on the effects of fungicides in the control of Alternaria disease, two spray rates each of Benlate and Daconil were tested on crambe plots during flowering and early seed-set. Seed weights were not significantly different, but seeds from plants receiving the high Daconil rate (2 pt/acre) had greater germination rates and seedling vigor, and lower incidence of Alternaria infection in the seedlings. Nitrogen applications increased seed weights and yield when applied at >60 lb/acre, but oil content of the seed and erucic acid levels in the oil were unaffected. Oil yields (lb/acre) were increased with N >60 lb/acre.

Publication:

PALMER, J. J. 1983. The effects of nitrogen applications on yield and oil characteristics of *Crambe abyssinica* (Hockst. ex. R. E. Fries) grown in western Kentucky. M.S. Thesis. Murray State University, Murray, Kentucky.

4. Long-Term and Endurance Engine Tests with Vegetable Oil Products as Diesel Fuel (Cooperative Agreement - North Dakota State University)

Engine tests were carried out under supervision of NDSU scientists at the Allis-Chalmers Engine Test Laboratory in Harvey, Illinois. Sunflower methyl esters and a blend of 25:75 high oleic safflower oil in diesel fuel were the only two fuels that passed the 200-hr EMA screening test. A nonionic sunflower oil-aqueous ethanol microemulsion developed at NRRC containing 53% sunflower oil survived the 200-hr test as far as power output and injector function were concerned, but fuel combustion was incomplete, carbon deposits were excessive, and lubricating oil viscosity increases were also excessive. A blend of 25:75 of sunflower oil in diesel oil without or with a barium smoke suppressant did not pass the test. A trial engine run with a high detergency fuel additive in the 25:75 blend indicated that this fuel might survive the test.

Publications:

ZIEJEWSKI, M., and KAUFMAN, K. R. 1983. Laboratory endurance test of a sunflower oil blend in a diesel engine. J. Am. Oil Chem. Soc. 60:1567-1573.

ZIEJEWSKI, M. 1983. Vegetable oils as a potential alternate fuel in direct injection diesel engines. SAE Paper No. 831359, Society of Automotive Engineers, Warrendale, PA.

ZIEJEWSKI, M., KAUFMAN, K. R., and PRATT, G. L. 1983. Alternative fuels for direct injection diesel engines. Proc. Vegetable Oils as Diesel Fuel, Seminar III, Northern Agricultural Energy Center, Peoria, IL. ARM-NC-28, pp. 106-111.

C. High Pressure Chemistry Research

J. P. Friedrich, Research Leader

1. Supercritical Fluid Technology for the Extraction of Seed Oils (J. P. Friedrich)

Triglycerides appear to be completely miscible with Supercritical CO₂ at temperatures above 80°C and pressures above 12,000 psi. Apparent solubility under extraction conditions is limited to the weight ratio of the available oil to CO₂ in the extraction vessel. Highly soluble, good flavored soybean flour can be produced by careful control of time, temperature, pressure and moisture during extraction. Deactivation of

peroxidase and lipoxygenase enzymes under the conditions of extraction contribute to improved flavor and storage stability. The morphology of SC-CO₂ extracted soy flakes is similar to hexane extracted flakes. The oils obtained by SC-CO₂ extraction of soybeans and cottonseed readily lend themselves to physical refining techniques because of their low phosphorus, free fatty acid and color. A computer program was designed to determine internal pressures of SC-CO₂ and other supercritical fluids from pressure-volume-temperature data. These internal pressures were then used to test the suitability of simplifying approximations involved in computing solubility parameters of these fluids from densities and critical pressures. These approximations are appropriate over a limited pressure range for nonpolar compounds but not for polar compounds.

Publications:

CHRISTIANSON, D.D., FRIEDRICH, J.P., BAGLEY, E.B., and INGLETT, G.E. 1982. Maize germ flours for food purposes by supercritical carbon dioxide extraction. In: "Maize: Recent Progress in Chemistry & Technology," ed. by G.E. Inglett Acad. Press, NY, pp. 231-239. (Book Chapter)

CHRISTIANSON, D.D., FRIEDRICH, J.P., LIST, G.R., WARNER, K., BAGLEY, E.B., STRINGFELLOW, A.C., and INGLETT, G.E. Supercritical extraction of dry-milled corn germ with carbon dioxide. J. Food Sci. (In Press).

FRIEDRICH, J.P. and LIST, G.R. 1982. Characterization of soybean oil extracted by supercritical carbon dioxide and hexane. J. Agric. Food Chem. 30:192-193.

FRIEDRICH, J.P., LIST, G.R., and HEAKIN, A.J. 1982. Petroleum-free extraction of oil from soybeans with supercritical CO₂. J. Am. Oil Chem. Soc. 59(7): 228-292.

FRIEDRICH, J.P. Supercritical CO₂ extraction of lipids from lipid-containing materials. Patent Serial #06/364,240.

FRIEDRICH, J.P. and CHRISTIANSON, D.D. 10/25/82. Production of food-grade germ product by supercritical fluid extraction. Patent Application #6916.

FRIEDRICH, J.P. and PRYDE, E.H. Supercritical CO₂ extraction of lipid-bearing materials and characterization of the products. J. Am. Oil Chem. Soc. (In press)

FRIEDRICH, J.P. and ELDRIDGE, A.C. 9/20/83. Production of defatted soybean products by supercritical fluid extraction. Patent Application #6922.

2. Concept Study of Continuous Countercurrent Supercritical Fluid Extraction of Vegetable Oil (Contract - Stanford Research Institute)

A series of meetings held at SRI International in Menlo Park, California, resulted in suggestions for three conceptual processes for continuous

extraction of oilseeds with supercritical carbon dioxide (SC-CO₂). In order of increasing feasibility, these processes were: (1) continuous, countercurrent extraction of oilseed pellets; (2) continuous, cocurrent extraction of pulverized oilseeds, followed by separation of the meal and extract, and washing of the meal on a solid bowl centrifuge; and (3) continuous, cocurrent extraction of pulverized oilseeds, followed by separation of the meal and extract, and washing of the meal on a filter. Other concepts for extraction with SC-CO₂ were recorded, and recommendations for research needed for more quantitative assessments of the feasibility of systems for continuous extraction on a commercial scale were categorized.

Publication:

PHILLIPS, R. C., and MURRAY, R. G. 1983. Technical feasibility of continuous extraction of oil from oilseeds with supercritical carbon dioxide. Final Report, SRI International Project No. PYC 4856. SRI Int., Menlo Park, Calif., 24 pp.

D. Lipid Chemistry Research

E. N. Frankel, Research Leader

1. Improving Food Quality of Vegetable Oils and Their Stability to Heat- and Light-Catalyzed Oxidation (E. N. Frankel)

An acetalation-acid decomposition procedure was developed to investigate the formation of malonaldehyde from a wide assortment of lipid oxidation products. Five-membered cyclic peroxides and 1,3-dihydroperoxides were found to be most important precursors of malonaldehyde. 1,4-Dihydroperoxides were less important, and monohydroperoxides were the least significant sources of malonaldehyde. No correlation was found between the thiobarbituric acid values of the lipid oxidation products and analyses of malonaldehyde. The specific methodology developed can be used to evaluate the potential of lipid oxidation products to form malonaldehyde and the biological effect due to crosslinking.

To elucidate the biological and flavor significance of lipid secondary oxidation products, cyclic peroxides and dihydroperoxides were prepared by photosensitized oxidation of methyl linolenate and its hydroperoxides. New secondary products identified included cyclic peroxides with one six-membered ring and bis-cyclic peroxides with one five- and one six-membered ring. Secondary products were decomposed either thermally or in the presence of HCl-methanol. The thermal decomposition products were those expected by homolytic fragmentation on both sides of the hydroperoxide group. The acid decomposition products were those expected by heterolytic cleavage only between the hydroperoxide group and the allylic double bond. Dialdehydes identified from acid decomposition of cyclic peroxides and dihydroperoxides included malonaldehyde and 3-hexenedial.

Publications:

- AWL, R. A., FRANKEL, E. N., and TJARKS, L. W. 1983. Cyclic fatty esters: Stereochemistry of monounsaturated products from hydrogenation and reduction of 9-(6-propyl-3-cyclohexenyl)-8-nonenic acid or ester. *Chem. Phys. Lipids*. In press.
- FRANKEL, E. N., NEFF, W. E., and SELKE, E. 1983. Analysis of autoxidized fats by gas chromatography-mass spectrometry. VIII. Volatile thermal decomposition products of hydroperoxy cyclic peroxides. *Lipids* 18:353-357.
- FRANKEL, E. N., and NEFF, W. E. 1983. Formation of malonaldehyde from lipid oxidation products. *Biochim. Biophys. Acta*. In press.
- FRANKEL, E. N., SMITH, L. M., HAMBLIN, C. L., CREVELING, R. K., and CLIFFORD, A. J. 1983. Occurrence of cyclic fatty acid monomers in frying oils used for fast foods. *J. Am. Oil Chem. Soc.* 61:87-90.
- FRANKEL, E. N. 1983. Recent advances in the chemistry of rancidity of fats. Proceedings of symposium on "Recent Advances in the Chemistry of Meat and Meat Products." Royal Society of Chemistry, London, England. In press.
- NEFF, W. E., FRANKEL, E. N., SELKE, E., and WEISLEDER, D. 1983. Photosensitized oxidation of methyl linoleate monohydroperoxides. Hydroperoxy-cyclic peroxides, dihydroperoxides, keto esters and volatile thermal decomposition products. *Lipids*. In press.

2. Quality Soybean and Cereal Foods for the Export Market (E. N. Frankel)

The effect of nonionic, anionic and amphoteric surfactants was investigated on the efficiency of aqueous degumming of oils from fresh and damaged beans with and without citric acid. With oils from normal beans, efficiency of citric acid degumming was improved by adding fatty alkyl oxazoline, polymeric sulfonate, alkyl sulfate and lecithin. With oils from damaged beans, although degumming was improved with nonionic and anionic surfactants the nonhydratable phosphatide content of the resulting oils was still too high, indicating the need for further processing. The phosphatide composition of crude oils from different quality beans was investigated by high-performance liquid chromatography. Oils from beans damaged by adverse storage for 16 and 18 days showed a decrease in phosphatidyl-ethanolamine and phosphatidyl choline, and an increase in phosphatidic acid and other unidentified components including unsaponifiable materials. The yield of oil from normal beans was varied by limiting hexane extraction to explore changes in phosphatide content and composition. Less total phosphatide was removed from the flakes by decreasing the volume of hexane extractant. This approach may provide oils that are easier to process and full-fat meals of higher nutritional content.

Publications:

NASH, A.M., KWOLEK, W.F., and MOUNTS, T.L. 1983. Determination of ultratrace metals in vegetable oils and fats. J. Am. Oil Chem. Soc. 60:811-814.

ANDERSON, R. A. 1983. Detoxification of aflatoxin-contaminated corn. In "Aflatoxin and Aspergillus flavus in Corn," U. L. Diener, R. L. Asquith, and J. W. Dickens, eds. South. Coop. Ser. Bull. 279:87-90. (Published at Alabama Agric. Exp. Sta., Auburn, Alabama).

PEPLINSKI, A. J., ECKHOFF, S. R., WARNER, K., and ANDERSON, R. A. 1983. Physical testing and dry milling of high-moisture corn preserved with ammonia while drying with ambient air. Cereal Chem. 60:442-445.

BOOKWALTER, G. N. 1983. World feeding strategies utilizing cereals and other commodities. Cereal Foods World 28:507-511.

BOOKWALTER, G. N. 1983. Cereal based foods for international feeding programs. In "Progress in Cereal Chemistry and Technology," J. Holas and J. Kratochvil, eds., Vol. 5B, p. 1153. (Elsevier Science Publishers, Amsterdam).

SELKE, E., and ROHWEDDER, W. K. 1983. Volatile components from trilinolenin heated in air. J. Am. Oil Chem. Soc. 60(11):1853-1858.

3. Heavy Metals in Soybeans Grown on Sewage Sludge-Amended Soil (Cooperative Agreement - Western Illinois University)

Sixty samples of soybeans have been received from scientists at Western Illinois University for trace metal analysis of oil and meal. Soybeans were grown during 1980, 1981, and 1982 on plots having 50 tons/acre or 100 tons/acre municipal sewage sludge applied either annually or during 1980 only. Yield data and metal analysis of soil and plant tissue were obtained. Soil samples were taken of the top 6 inches of soil, while tissue samples were taken by removing three leaflets near the top of each plant. Significant increases in soil metal content were detected, as expected, with increasing level and frequency of sludge application. There was no significant correlation between sludge application and metal analysis of plant tissue. Yield data was variable and impacted by weed growth on sludge treated plots.

Publications:

ROSKAMP, G. 1980. Effects of sewage sludge amended soil on trace metal content of soybeans, oil, and meal. Fifth Annual Report of Research, Western Illinois Univ., Agricultural Experiment Station, Macomb, pp. 27-28.

ROSKAMP, G. 1981. Effects of sewage sludge amended soil on trace metal content of soybeans, oil, and meal. Sixth Annual Report of Research, Western Illinois Univ., Agricultural Experiment Station, Macomb, pp. 27-29.

ROSKAMP, G. 1982. Effects of sewage sludge amended soil on trace metal content of soybeans, oil, and meal. Seventh Annual Report of Research, Western Illinois Univ., Agricultural Experiment Station, Macomb, pp. 55-57.

E. Meal Products Research

W. J. Wolf, Research Leader

1. Analytical and Structure Studies of Soybean Proteins (W. J. Wolf)

Commercial soy phosphatidylcholine was converted into liposomes in the presence of ^{14}C -labelled sucrose; $19 \pm 5\%$ was incorporated. The liposomes were stable for 1-2 weeks in the cold. Addition of Fe^{+3} caused a significant decrease in the amount of entrapped ^{14}C sucrose. Addition of soy 7S and 11S proteins also reduced the level of entrapped sucrose. Analytical studies on fate of nightshade alkaloids during processing of contaminated soybeans revealed that the alkaloids remain in the meal during hexane extraction. When processed into protein concentrates and isolates, the alkaloids in contaminated flakes were found in all fractions except a protein concentrate made by alcohol extraction. A variety of soybean protein fractions have been prepared and used to evaluate variables in development of an HPLC method for analyses of soy proteins. A strategy was developed for separation, identification and quantification of trypsin inhibitors in raw soy flour for comparison with residual trypsin inhibitor activity in toasted soy flour. Titrimetric and colorimetric trypsin inhibitor assays have been compared. Gelatin has been used as a model system to evaluate rheological measurements on gels. Gels were subjected to simple shear, orthogonal rheometry and torsion measurements in a mechanical spectrometer and $(C_1 + C_2)$, the sum of two parameters for a Mooney-Rivlin material was calculated. Simple shear and orthogonal rheometry gave good agreement in $(C_1 + C_2)$ whereas torsion data were variable.

Publications:

CHRISTIANSON, D. D., NAVICKS, L. L., BAGLEY, E. G., and WOLF, W. J. Rheological characterization of starch, starch-hydrocolloid and protein dispersions and gels in simple shear and torsion. Prog. Food Nutr. Sci. In press.

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ELDRIDGE, A. C., and HOCKRIDGE, M. E. High-performance liquid chromatographic separation of Eastern Black Nightshade (Solanum ptycanthum) glycoalkaloids. J. Agric. Food Chem. In press.

ELDRIDGE, A. C., and KWOLEK, W. F. 1983. Soybean isoflavones: Effect of environment and variety on composition. J. Agric. Food Chem. 31:394-396.

WARNER, K., MOUNTS, T. L., RACKIS, J. J., and WOLF, W. J. 1983. Relationships of sensory characteristics and gas chromatographic profiles of soybean protein products. *Cereal Chem.* 60:102-106.

WANG, L. C., and WOLF, W. J. 1983. Soybean protein aggregation by sonication: Ultracentrifugal analysis. *J. Food Sci.* 48:1260-1264.

WOLF, W. J. 1983. Soybeans and other oilseeds. In *Encyclopedia of Chemical Technology* 21:417-441.

WOLF, W. J. 1983. Research needs for a new solvent to replace n-Hexane in soybean oil extraction. Soybean research in China and the United States. Proceedings of the First China/USA Soybean Symposium and Working Group Meeting International Soybean Program, University of Illinois, INTSOY Series No. 25, 114-116.

WOLF, W. J. 1983. Edible soybean protein products, *CRC Handbook of Processing and Utilization in Agriculture*, Vol. II, Part 2 Plant Products, I. A. Wolff, Ed., CRC Press, Inc., Boca Raton, Florida, pp. 23-55.

WOLF, W. J. Present status of edible soybean products in the United States. Proceedings of United States/People's Republic of China Symposium and Workshop. In press.

2. Nutritional Quality, Safety and Flavor Aspects of Soybean Protein Products (J. J. Rackis)

A 2-year feeding study on the biochemical effects of soy protein isolates on the rat pancreas was completed in August. No significant change in TI activity, protein and moisture content of the isolates occurred during 2-year cold storage. Protocols and diets have been prepared for a similar study on potato and soy trypsin inhibitors (TI). Pilot-plant procedures were developed for efficient extraction of TI concentrate from defatted soy flour. TI concentrate (42 lb) with a purity of 35% was prepared for 2-year rat feeding trials. TI activity was present in supernatant and precipitate fractions of extracts of toasted soy flour treated with 0.8 N Trichloroacetic acid. Bowman-Birk TI was found in both fractions, only the precipitate contained the Kunitz TI. Phytic acid content of the 11S and 7S components, soy protein isolate and its pH 8.0 insoluble fraction ranged in increasing order from 0.1 to 1.41 percent, respectively. A purified 11S was prepared for microcolorimetric studies. Isopropyl alcohol extracted soybean flakes have been prepared for digestion metabolism studies on lambs at University of Illinois. Chemical ionization mass spectrometry (CI/MS) of phospholipids (PL) volatilized from a direct exposure probe provided, in less than a minute, spectra from which compound molecular species could be quantitated. CI/MS spectra of PL standards gave molecular weight response factors and correction factors for isotope effect and H abstraction. Internal standards allowed absolute quantitation. Different species with the same molecular weight were quantified by tandem MS daughter ion analysis. CI/MS was also used to identify some oxidized PL.

Publications:

GARNER, H. W., and CRAWFORD, C. G. 1983. Negative Ames tests of epoxide fatty esters derived from homolysis of linoleic acid hydroperoxides. Food Chem. Toxic. 21(2):175-180.

CRAWFORD, C. G., and PLATTNER, R. D. 1983. Ammonia chemical ionization mass spectrometry of intact diacyl phosphatidyl-choline. J. Lipid Res. 24:456-460.

HONIG, D. H., HOCKRIDGE, M. E., GOULD, J. M., and RACKIS, J. J. 1983. Determination of cyanide in soybeans and soybean products. J. Agric. Food Chem. 31:272-275.

BAKER, E. C., and BROWN, R. L. 1983. A coalescer for soybean oil emulsions. J. Am. Oil Chem. Soc. 60:851.

BAKER, E. C., and SULLIVAN, D. A. 1983. Development of a pilot-plant process for the extraction of soybean flakes with aqueous isopropyl alcohol. J. Am. Oil Chem. Soc. 60:1271-1277.

3. Effects of Vegetable and Animal Trypsin Inhibitors in Long-Term Animal Feeding Studies (Cooperative Agreement - University of Minnesota)

Antibody fractions (IgG) have been prepared from antisera of pancreatic tissue with hyperplastic nodules from rats fed raw soy flour and normal tissue from rats fed casein diets. Gel electrophoresis revealed that the antigen fraction (AgT) of the tumor tissue contained 9 components not present in the antigen fraction (AgN) of normal pancreas. Ouchterlony immunodiffusion showed that only 2 of the components of AgT produced precipitation lines with IgG isolated from antisera of tumor tissue and none with IgG from antisera of normal tissue. Therefore, the AgT preparation contains at least 2 antigens specific for tumor pancreatic tissue. Four week studies reveal that raw soy flour diets inhibited growth and caused pancreatic enlargement in mice, hamsters, and rats. However, pancreatic hyperplastic nodules and adenomas were found in rats, but not in mice and hamsters after about 16 months on raw soy diets.

Publications:

HASDAI, A., and LIENER, I. E. 1983. Growth, digestibility, and enzymatic activities in the pancreas and intestines of hamsters fed raw and heated soy flour. J. Nutr. 113:662-668.

LIENER, I. E. 1983. Naturally occurring toxicants in foods and their significance in the human diet. Arch. Toxicol. 6:153-166.

NITSAN, Z., and LIENER, I. E. 1983. Accentuated response to raw soya bean meal by meal feeding. Arch. Toxicol. 6:177-181.

4. Effects of Dietary Soy Protein on Metabolic Processes in Subhuman Primates
(Cooperative Agreement - New England Regional Primate Research Center)

Biochemical and histopathological evaluations were conducted on 27 juvenile Cebus monkeys which had been reared from infancy on semi-purified liquid and agar-cake diets containing 12% (as percent of calories) lactalbumin (n=8) trypsin inhibitor (TI) content (mg/g) = 0.64; 20% soy isolate (n=10) TI = 2.70; 13% casein (n=7) TI = 0.60 or 20% soy concentrate (n=2) TI = 7.90. Complete hematological and serum clinical chemistry analyses of each animal revealed no important differences in group means. Examination of pancreatic tissue by histopathological evaluation and determination of protein, RNA and DNA content, and trypsin and chymotrypsin activity showed no differences in animals fed lactalbumin, soy isolate and casein. Too few surviving animals were on soy concentrate diets to obtain significant data; one of the two animals showed some diffuse fibrosis of the pancreas which apparently resulted in changes in biochemical evaluations. The other animal on soy concentrate showed no significant differences from test animals on other diets. The diets appear to provide for adequate health and development of the animal and to have no adverse effects on the pancreas.

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